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Poster

581. Modeling Neurodegenerative Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 581.01/A1

Topic: A.03. Stem Cells and Reprogramming

Support: VA Merit Award I01BX002452

NYSTEM contract C028129

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Title: Cell cycle and p53 gate the direct conversion of human fibroblasts to dopaminergic neurons.

Authors: *J. FENG[1], H. JIANG[1], Z. XU[1], P. ZHONG[1], Y. REN[1], G. LIANG[2], H. SCHILLING[1], Z. HU[1], Y. ZHANG[2], X. WANG[3], S. CHEN[4], Z. YAN[1];
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Abstract: The direct conversion of fibroblasts to induced dopaminergic (iDA) neurons and other cell types demonstrates the plasticity of cell fate. The low efficiency of these relatively fast conversions suggests that kinetic barriers exist to safeguard cell type identity. Here we show that suppression of p53, in conjunction with cell cycle arrest at G1 and appropriate extracellular environment, markedly increase the efficiency in the transdifferentiation of human fibroblasts to iDA neurons by Ascl1, Nurr1, Lmx1a and miR124. The conversion is dependent on Tet1, as G1 arrest, p53 knockdown or expression of the reprogramming factors induces Tet1 synergistically. Tet1 knockdown abolishes the transdifferentiation while its overexpression enhances the conversion. The iDA neurons express markers for midbrain DA neurons, support dopaminergic transmission, and extend arborization and synaptic connections when grafted in rat brains. Our results suggest that overcoming these kinetic barriers may enable highly efficient epigenetic reprogramming in general and will generate patient-specific midbrain DA neurons for Parkinson's disease research and therapy.

Disclosures: **J. Feng:** None. **H. Jiang:** None. **Z. Xu:** None. **P. Zhong:** None. **Y. Ren:** None. **G. Liang:** None. **H. Schilling:** None. **Z. Hu:** None. **Y. Zhang:** None. **X. Wang:** None. **S. Chen:** None. **Z. Yan:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 581.02/A2

Topic: A.03. Stem Cells and Reprogramming

Support: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 668738.

Title: Highly efficient derivation of human midbrain neuroepithelial stem cells and dopaminergic neurons from pluripotent stem cells by mimicking embryonic midbrain development

Authors: *K. HEMMER, J. C. SCHWAMBORN;
Developmental and Cell. Biol., Luxembourg Ctr. For Systems Biomedicine, Belvaux, Luxembourg

Abstract: Parkinson´s disease (PD) is the second most common neurodegenerative disorder characterised by non-motor symptoms like hyposmia or depression, and motor symptoms like resting tremor, bradykinesia, akinesia, rigidity and postural instability. The progressive loss of midbrain dopaminergic neurons (mDANs) of the substantia nigra pars compacta is the main pathology causing these motor symptoms. Conventional therapies only achieve temporary symptomatic relief. However, no neuroprotective treatment exists yet that halts or even counteracts the progression of the disease. Consensus is growing that this failure of bench-to-bedside translation can be attributed to the lack of appropriate preclinical PD models. The generation of patient-specific mDANs offers an attractive tool to model PD *in vitro*. This novel model can be used to further understand disease processes, and to screen for compounds for neuroprotective therapies. For such approaches it is a requirement that *in vitro*-derived mDANs are as similar as possible to their *in vivo* counterparts. This similarity includes the expression of midbrain specific markers, electrophysiological functionality, morphology, and dopamine production. During embryonic development, the patterning of mDANs is a carefully balanced process that is defined by a precise timing of inhibiting or inductive cues produced by organising centres. In the here described project, we established a novel highly efficient mDANs differentiation protocol by modulating developmentally regulated pathways, similar to the *in vivo* mDANs differentiation process. In our novel protocol human induced pluripotent stem cells

(iPSCs) are regionalized toward a midbrain neuroepithelial stem cell (mNESCs) fate by mimicking sonic hedgehog (SHH) signalling, wingless-type MMTV integration site (WNT) signalling and the fibroblast growth factor (FGF) pathway. This derivation process is conducted under hypoxic conditions. The thereby obtained mNESCs are able to self-renew and can be efficiently differentiated into tyrosine hydroxylase (TH) positive dopaminergic neurons. Moreover, immunostainings of differentiated neurons reveal the expression of the midbrain markers forkhead box A2 (FOXA2), LIM homeobox transcription factor 1 (LMX1), nuclear receptor subfamily 4 group A member 2 (NR4A2, also known as NURR1), and engrailed 1 (EN1). We conclude that our novel protocol for *in vitro* derivation of midbrain specific dopaminergic neurons mimics the according *in vivo* developmental process. This new protocol represents an attractive starting point for the utilization of patient-specific cells for *in vitro* modelling of PD.

Disclosures: K. Hemmer: None. **J.C. Schwamborn:** A. Employment/Salary (full or part-time): Braingineering Technologies s.a.r.l..

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 581.03/A3

Topic: A.03. Stem Cells and Reprogramming

Support: NIH/NINDS; R01 NS085011

Title: Developing iPSC-derived neurons for studying the regulation of SNCA expression by miRNAs in relation to Parkinson's and Dementia with Lewy body diseases.

Authors: *L. TAGLIAFIERRO, M. W. LUTZ, O.-C. GLENN, O. CHIBA-FALEK; Neurol., Duke Univ. Hlth. Syst., Durham, NC

Abstract: Synucleinopathies are a group of neurodegenerative diseases that share a common pathological lesion of intracellular aggregates, named Lewy Bodies, composed mainly of α-synuclein protein encoded by the *SNCA* gene. Genetic studies have implicated *SNCA* in the etiology of these diseases. This study focuses on two diseases in this group: Parkinson's Disease (PD) and Dementia with Lewy Bodies (DLB). Both are characterized by α-synuclein aggregates in the neurons, however they have distinct characteristics with respect to the cell type containing the aggregates and the predominantly affected brain region. Specifically, dopaminergic neurons are primarily affected in PD whereas DLB is defined by aggregates in cholinergic neurons. Our goal is to explore common and distinct regulatory mechanisms of *SNCA* gene expression in the

context of these pathologies. It has been suggested that *SNCA* expression levels are critical for the development of synucleinopathies. Gene expression levels are affected by different factors: genetic, epigenetic, environment, gender, and age. Our lab aims to explore the genetic regulatory mechanisms underlying *SNCA* expression levels that include *cis* and *trans*-acting factors. This work focuses on the regulation of SNCA by microRNAs (miRNA). Towards this goal, we established a model system of induced Pluripotent Stem Cell (iPSC)-derived neurons from a subject with normal Karyotype and from a patient with *SNCA*-triplication. These two iPSC lines were differentiated into dopaminergic and cholinergic neurons to model PD and DLB, respectively, and the analysis of miRNAs was performed for each neuronal type at four differentiation stages: iPSCs, Neural Precursor Cells, final neurons, and aged neurons. miRNA are known to regulate gene expression via interactions with Untranslated Regions (UTRs). A computational analysis using TargetScan identified five miRNA binding sites in *SNCA* 3'UTR. Evaluation of the miRNA expression profiles revealed distinctive pattern between the dopaminergic and the cholinergic neurons and along the differentiation stages of each neuronal type. *SNCA* mRNA levels have been measured at all differentiation points of the dopaminergic and cholinergic neurons, and were correlated with the miRNA profiles. Experiments of genome editing using CRISPR/Cas9 technology are underway to evaluate how *cis*-variants modulate *SNCA* expression via their interactions with the *trans*-acting factors. In conclusion, we developed an isogenic iPSC-based model system to better understand the *cis* and *trans* acting factors that contribute to the regulation of *SNCA* expression in the context of PD and DLB.

Disclosures: L. Tagliafierro: None. **M.W. Lutz:** None. **O. Glenn:** None. **O. Chiba-Falek:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 581.04/A4

Topic: A.03. Stem Cells and Reprogramming

Title: Multielectrode array platform to study long-term potentiation in human induced pluripotent stem cell-derived neuronal networks

Authors: *S. BIESMANS, S. HINCKLEY, A. BANG;
CPCCG Screening Ctr., Sanford Burnham Prebys Med. Discovery Inst., LA Jolla, CA

Abstract: Numerous neurological conditions including neurodegenerative and psychiatric disorders such as Alzheimer's disease and clinical depression are characterized by synaptic dysfunction and cognitive impairment. Long-term potentiation (LTP) is a persistent

strengthening of synapses between neurons that is considered to be one of the major cellular mechanisms underlying memory and learning. Disease modeling and drug discovery approaches would benefit tremendously from human cell-based platforms to study LTP in disease. Human induced pluripotent stem cells (hiPSC) offer such a system in that they circumvent issues of species specificity, are scalable, and can carry complex genetic backgrounds, which is especially important for modeling diseases with high heritability. Here we present a platform to study LTP in neuronal networks comprised of hiPSC-derived neurons. Using either iCell® DopaNeurons or in-house generated hiPSC-derived cortical neurons co-cultured with human primary astrocytes, we developed a procedure that supports neuronal network formation on 48-well multielectrode arrays. Analysis of spontaneous electrical activity shows that an initial phase of primarily single spike firing is followed by synchronized bursting indicating development of functional neuronal networks. At 4 weeks after plating, 84% of the electrodes recorded spontaneous synchronized bursting events wherein most of the neurons in the network spiked together. At this point, the cultures were exposed to forskolin and rolipram to trigger LTP by increasing intracellular cyclic adenosine monophosphate levels, resulting in a significant increase in the number of network bursts that lasted up to 7 days after washing off the drugs. Long-lasting maintenance of LTP requires *de novo* gene expression and protein synthesis. Ongoing studies include expression analyses of transcription factors and signaling molecules that regulate enduring LTP. In conclusion, we have developed a robust, medium-throughput platform to study LTP in hiPSC-derived neuronal networks. This technology will allow for disease modeling and drug screening of disease-relevant cell types on patient-specific genetic backgrounds.

Disclosures: **S. Biesmans:** None. **S. Hinckley:** None. **A. Bang:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

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Topic: A.03. Stem Cells and Reprogramming

Support: Institutional funds from Children's Mercy Hospital

Ron D. Deffenbaugh Foundation

Title: Survival and development of different subtypes of neural progenitor cells transplanted into jaundiced and non-jaundiced rat brain

Authors: ***F.-C. YANG**[1], S. M. RIORDAN[4], M. WINTER[2], L. GAN[1], J. L. VIVIAN[3], J. A. STANFORD[1,2], S. M. SHAPIRO[4];

[1]Dept. of Mol. and Integrative Physiol., [2]Kansas Intellectual & Developmental Disabilities Res. Ctr., [3]Dept. of Pathology & Lab. Med., Univ. of Kansas Med. Ctr., Kansas City, KS; [4]Div. of Child Neurology, Dept. of Pediatrics, Children's Mercy Hosp. & Clinics, Kansas City, MO

Abstract: Neonatal hyperbilirubinemia targets specific brain nuclei and can lead to kernicterus. One of the most vulnerable brain regions is basal ganglia, especially the globus pallidus (GP). A debilitating symptom of kernicterus associated with GP damage is dystonia. Stem cell transplantation has been suggested to effectively improve motor function in basal ganglia-related diseases such as Parkinson's disease and Huntington's disease. We believe that targeting affected brain regions with neuronal stem cells is a promising therapeutic approach for treating dystonia in kernicterus. It is unknown, however, how elevated bilirubin levels in the brain will affect neural progenitor cell survival and functional development. In this study, we injected different subtypes of neural progenitor cells (resemble excitatory spinal cord interneurons or inhibitory basal ganglia neurons) into the basal ganglia of jaundiced (jj) Gunn rats and their nonjaundiced (Nj) littermates and compared cell survival, fiber outgrowth, and cell properties. Each animal received 10,000 neural progenitor cells (2.5µl) injected unilaterally at P20. Animals were perfused at P43 and 30 µm of brain sections through the injection site and the GP were collected for immunohistochemical (IHC) analyses for STEM121 (human cells), Ku80 (human cell nucleus), and hSYP (human-specific synaptophysin) expression. Our preliminary results indicated, 3 weeks after transplantation, neural precursor cells survived and migrated outside the injection sites in the striatum of both jj and Nj rats. Grafted neurons frequently formed abundant neurites. Some neurites extended toward the GP or cerebral cortex. Very long fibers traveling along the corpus callosum were also observed. However, there were only low levels of hSYP-ir staining identified on/nearby the outgrowth fibers of both jj and Nj brains, suggesting that synaptogenesis is impaired or still developing. We conclude that neural precursor cells not only survive within the basal ganglia of jaundiced rats, but they also form neurites. These results support the feasibility of neural stem cell injections for the treatment of kernicterus. Future investigation into the use of these cells as a possible treatment of dystonia due to kernicterus will focus on alternative stem cells lines with larger percentages of GABAergic and cholinergic neurons to more closely mimic the major cell types of the GP.

Disclosures: F. Yang: None. **S.M. Riordan:** None. **M. Winter:** None. **L. Gan:** None. **J.L. Vivian:** None. **J.A. Stanford:** None. **S.M. Shapiro:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

Location: Halls B-H

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Program#/Poster#: 581.06/A6

Topic: A.03. Stem Cells and Reprogramming

Title: Direct induction of human motor neuron progenitors from peripheral blood of patients with primary lateral sclerosis

Authors: *T. WANG, M. MEDYNETS, M. K. FLOETER, A. NATH;
NINDS/NIH, Bethesda, MD

Abstract: Primary lateral sclerosis (PLS) is a neuromuscular disorder characterized by progressive weakness of voluntary muscles caused by the chronic degeneration of upper motor neurons in the central nervous system. The pathogenesis of PLS is largely unknown and its study is hindered by the lack of access of patient specific motor neurons which is pivotal for constructing in vitro disease models. Recent developments in the field of cell transformation make it possible to derive neurons from induced pluripotent stem cells which can be generated by transfecting other somatic cells with Yamanaka factors. However, generating iPSC is a time and labor demanding process and requires expertise in the field. We recently reported generating neural stem cells directly using peripheral blood cells which are clinical available. Here we aimed to directly generating motor neuron progenitor cells from clinical blood samples which can provide a large quantity of motor neurons for the in vitro study of motor neuron disorders. By transfecting enriched CD34+ cells from patient blood samples with Sendai virus containing Yamanaka factors (Oct4, Sox2, C-Myc and Klf4), we collected cells after five days of culture and transferred them to motor neuron progenitor cells media for another two weeks to make neural spheres. For motor neuron differentiation, the resulting spheres were then cultured on matrigel coated plates in motor neuron differentiation media for another 10 days. The resulting spheres and neurons were further immunostained for neural progenitor and motor neuron markers such as Nestin, HB9, ISL-1 and ChAT. We found that nestin-positive neural spheres were formed as early as one week after the neural induction and HB9 and ISL-1 positive spheres were formed two weeks after. These neurospheres were differentiated into ChAT positive motor neurons when cultured in motor neuron differentiation media. The whole process takes about a month and we believe this is a convenient and the fastest technique to generate motor neurons directly form clinical blood samples. The generated motor neuron progenitors could be very useful for in vitro modeling of motor neuron diseases such as PLS and potentially for developing neural transplantation therapies.

Disclosures: **T. Wang:** None. **M. Medynets:** None. **M.K. Floeter:** None. **A. Nath:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

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Topic: A.03. Stem Cells and Reprogramming

Support: E-rare

Title: Increased susceptibility of machado joseph disease patient-specific neurons to stress

Authors: *J. JATHO-GRÖGER, K. KLEINSIMLINGHAUS, D. POPPE, J. LADEWIG, P. KOCH;
Inst. of Reconstructive Neurobio., Bonn, Germany

Abstract: Machado Joseph Disease (MJD)/ Spinocerebellar Ataxia Type 3 (SCA3) belongs to the group of polyglutamine (polyQ) expansion disorders and is the most prevalent autosomal dominantly inherited cerebellar ataxia worldwide. The cause of this progressive neurodegenerative disease is an expansion of a CAG trinucleotide repeat in exon 10 of the MJD1 gene leading to an extended tract of repeated glutamines (polyQ) in the resulting Ataxin3 protein (ATXN3). Recent data suggest that either conformational changes of the expanded ATXN3 protein and/or a loss of free available ATXN3 due to recruitment of ATXN3 into aggregates leads to the dysregulation of multiple cellular pathways such as ubiquitination or transcriptional regulation. Here we used induced pluripotent stem cells (iPSCs) to study the influence of expanded and/or lost ATXN3 on gene expression in patient-specific neurons. To that end we generated isogenic iPSC-derived neural stem cell lines, expressing either the normal (ATXN3cont), the expanded (ATXN3exp) or no ATXN3 (ATXN3ko) using CRISPR/Cas9-mediated gene editing. By transcriptional profiling of neurons generated from these lines we identified several genes, which were dysregulated in ATXN3exp and ATXN3ko neurons. Among those we identified a cluster of genes, which was significantly downregulated in ATXN3exp and ATXN3ko neurons compared to their isogenic controls. Functionally, these genes are important regulators of cell homeostasis and participate in an array of protective stress responses. Consequently, we found that ATXN3exp and ATXN3ko neurons are more susceptible to several types of cellular stress. Our findings support that ATXN3 has an important role in regulating the cellular stress response and that an increased susceptibility towards stress contributes to the pathogenesis of Machado Joseph Disease.

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Poster

581. Modeling Neurodegenerative Disease from iPS Cells

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Topic: A.03. Stem Cells and Reprogramming

Support: Department of Defense CDMRP W81XWH-15-1-0187

NIH/NINDS R00NS077435

New York Stem Cell Foundation-Robertson Investigator Award

Title: Identification of therapeutic targets and pathogenic mechanisms for C9orf72 ALS using phenotypic chemical screening

Authors: *J. ICHIDA, Y. SHI, S. LIN, Y. LI, K. STAATS, E. SON;
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Abstract: Expansion of a GGGGCC repeat in *C9orf72* recently emerged as the most common cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), making it a key therapeutic target. However, the neurodegenerative mechanisms underlying *C9orf72* ALS/FTD are unclear, limiting the identification of therapeutic strategies. To identify potential therapeutic targets and delineate pathogenic mechanisms, we established a model of *C9orf72* ALS using induced motor neurons (iMNs) generated by transcription factor-based reprogramming and performed a chemical genetic screen using neuron survival as a readout. *C9orf72* patient iMNs, but not induced dopaminergic neurons, undergo accelerated degeneration (3 patients, 3 controls, p=.002) and possess the hallmark pathology of *C9orf72* ALS. Removing the repeat expansion CRISPR/Cas9 editing fully rescues iMN survival. Thus, *C9orf72* iMNs faithfully model ALS disease processes. From a screen of 1000 bioannotated small molecules, we identified three classes of molecules that rescue *C9orf72* patient iMN survival without affecting the survival of controls, as well as one class that specifically accelerates *C9orf72* iMN degeneration. We find that at least one class of therapeutic compounds rescues *C9orf72* iMN survival by reversing a defect in endocytosis that causes aberrant upregulation of glutamate receptors on iMNs. By restoring proper glutamate receptor homeostasis, which is maintained in neurons through endocytosis, this class of molecules prevent *C9orf72* iMN degeneration by excitotoxicity, which we find to be the major cause of neurodegeneration in our model. Verifying the relevance of these findings to patients, glutamate receptors are significantly upregulated on motor neurons in postmortem tissue from both patients and *C9orf72* mutant mice, and neurons in *C9orf72* mice undergo hyperactivation when exposed to glutamate *in vivo*. Thus, our results highlight the importance of early endosomal trafficking in *C9orf72* ALS pathogenesis, and identify several potential therapeutic targets.

Disclosures: **J. Ichida:** None. **Y. Shi:** None. **S. Lin:** None. **Y. Li:** None. **K. Staats:** None. **E. Son:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

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Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant R21MH096233

NIH Grant R33AG049864

Title: Single-cell detection of secreted Aß and sAPPα secreted from human iPSC-derived neurons and astrocytes

Authors: ***M.-C. LIAO**[1,2], C. MURATORE[2], T. GIERAHN[3], S. SULLIVAN[2], P. SRIKANTH[2], P. DE JAGER[2], J. LOVE[3], T. YOUNG-PEARSE[2];
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Abstract: Secreted factors play a central role in normal and pathological processes in every tissue in the body. The brain is composed of a highly complex milieu of different cell types, and few methods exist that can identify which individual cells in a complex mixture are secreting specific analytes. By identifying which cells are responsible, we can better understand neural physiology and pathophysiology, more readily identify the underlying pathways responsible for analyte production, and ultimately use this information to guide the development of novel therapeutic strategies that target the cell types of relevance. We present here a method for detecting analytes secreted from single human iPSC-derived neural cells, and have applied the method to measure Aβ and sAPPα, analytes central to Alzheimer's disease pathogenesis. Through these studies, we have uncovered the dynamic range of secretion profiles of these analytes from single iPSC-derived neuronal and glial cells, and have molecularly characterized subpopulations of these cells through immunostaining and gene expression analyses. In examining Aβ and sAPPα secretion from single cells, we were able to identify previously unappreciated complexities in the biology of APP cleavage that could not otherwise have been found by studying average responses over pools of cells. This technique can be readily adapted to the detection of other analytes secreted by neural cells, which would have the potential to open new perspectives into human CNS development and dysfunction.

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Poster

581. Modeling Neurodegenerative Disease from iPS Cells

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Topic: A.03. Stem Cells and Reprogramming

Support: NIH/NINDS (U54NS091046-01)

ALS Association

Title: Transcriptional comparisons of motor neuron cellular models reveal gene networks associated with maturation, age, and ALS

Authors: *R. HO, S. SANCES, C. N. SVENDSEN;
Cedars-Sinai Med. Ctr., Los Angeles, CA

Abstract: Modeling Amyotrophic Lateral Sclerosis (ALS) with human induced pluripotent stem cells (iPSCs) or with transgenic mice aims to reenact embryogenesis, maturation, and aging of motor neurons (MNs) *in vitro*. Studies have identified molecular pathways affected by ALS conditions in both model systems as well as in post-mortem human MNs. However, the extent to which mouse ALS models faithfully recapitulate pathways observed in human MNs has not been closely examined. Additionally, the maturity of MNs grown *in vitro* compared to MNs *in vivo* remains largely unaddressed; therefore, it is unclear to what extent this *in vitro* system captures critical aspects of MN development and molecular signatures associated with ALS. We compared transcriptomes among human iPSCs, iPSC-derived MNs (iMNs), fetal MNs, and adult MNs, as well as orthologous tissues from mice. Principal component analysis produced a maturation scale revealing that iMNs were more similar to fetal tissue than to adult MNs. Additionally, we resolved gene networks and pathways associated with MN maturation and aging. During maturation, expression increased for axonogenesis, myelination, synaptic transmission, and immune response pathway genes and expression decreased for translation, mitochondrial electron transport, and DNA repair pathway genes. During aging, expression increased for proteasomal genes and, interestingly, expression decreased for immune response and amyloid catabolism genes. These networks enriched for familial ALS genetic variants and were also affected in familial as well as sporadic ALS MNs. Based on these principal component and gene co-expression network analyses, we developed a panel of twenty key genes that can accurately assess either the maturation or disease state of cell or tissue samples. Genetic and

epigenetic analysis of regulatory features associated with co-expression networks revealed candidate pre- and post-transcriptional regulators of co-expressed genes that could be targeted to affect MN development, maturation, and disease. Lastly, we demonstrate that the global expression of orthologous genes involved in spinal cord MN fetal development and maturation are largely parallel between human and mouse systems, and gene co-expression networks associated with maturation are well-conserved between the two species. This analysis thus provides a more sensitive interrogation of gene-to-gene expression relationships underlying human and mouse spinal cord MN physiology. Altogether, our findings suggest that developing strategies to further mature and age iPSC-derived MNs will provide more effective iPSC models of ALS.

Disclosures: **R. Ho:** None. **S. Sances:** None. **C.N. Svendsen:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

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Topic: A.03. Stem Cells and Reprogramming

Support: ALS Association 11000 GE232

Title: C9orf72 als patient and control ipsc line-derived cortical neurons and astrocytes reveal diminished network activity when co-cultured with c9orf72 patient-derived astrocytes

Authors: *V. J. GARCIA, G. M. THOMSEN, D. RUSHTON, K. WU, R. H. BALOH, C. N. SVENDSEN;
Cedars-Sinai, West Hollywood, CA

Abstract: Amyotrophic lateral sclerosis (ALS) is characterized by the loss of neurons in the cortex, brain stem and spinal cord, and also by the malfunction of astrocytes. Only ~10% of ALS diagnoses are attributed to a genetic mutation. A recently discovered mutation on chromosome 9 open reading frame 72 (C9orf72) has been connected to approximately 40% of familial ALS and 9% of sporadic ALS disease instances. This novel mutation provides a valuable opportunity to investigate ALS disease mechanisms. Here, we explored the physiology of astrocyte and cortical neuron co-cultures that were differentiated from C9orf72 patient-derived induced pluripotent stem cell (iPSC) lines or from control iPSC lines. Using microelectrode array, we evaluated combinations of disease or control astrocyte and neuron co-cultures, and found that astrocytes derived from control iPSC lines promote healthier network activity in both control and C9orf72-derived neurons, when compared to diseased astrocytes or neurons alone. Using patch-clamp, we

describe intrinsic, physiological properties of diseased astrocytes and cortical neurons. Further, we use two C9orf72 isogenic lines to evaluate the rescue of disease phenotypes.

Disclosures: **V.J. Garcia:** None. **G.M. Thomsen:** None. **D. Rushton:** None. **K. Wu:** None. **R.H. Baloh:** None. **C.N. Svendsen:** None.

Poster

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Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant EY024984

BrightFocus Foundation Grant G201027

Title: Functional maturation and *In vitro* disease modeling of human pluripotent stem cell-derived retinal ganglion cells

Authors: ***J. S. MEYER**[1], S. K. OHLEMACHER[2], C. M. FLIGOR[2], A. SRIDHAR[2], Y. XIAO[3], T. R. CUMMINS[4];
[1]Dept. of Biol., [2]Biol., IUPUI, Indianapolis, IN; [3]Stark Neurosciences Res. Inst., [4]Pharmacol. and Toxicology, Indiana Univ., Indianapolis, IN

Abstract: Retinal ganglion cells (RGCs) play an essential role in transmitting visual information from the eye to the visual thalamus in the brain. They are also the primary cell type affected in traumatic retinal injuries as well as optic neuropathies. Human pluripotent stem cells (hPSCs) provide an attractive source of cells that can be used for patient-specific cell replacement therapies, drug screening, and disease modeling. However, little is known about the functional maturation of these hPSC-derived RGCs. As such, the development of these neurons from hPSCs was evaluated over time, and methods in which to influence their maturation and functionality were tested. hPSCs were differentiated toward a neural lineage in a stepwise fashion as previously described, yielding highly enriched populations of optic vesicle-like neurospheres. Within 40 days of differentiation, presumptive RGCs differentiated in a temporally-appropriate manner and expressed a compliment of RGC-associated features as well as proper morphological features. In prolonged culture, subtypes of RGCs emerged over time, including melanopsin-expressing intrinsically photosensitive RGCs. The maturation of hPSC-derived RGCs in extended cultures was observed by extensive neurite outgrowth, and the development of synaptic-like structures was further examined by microscopy and

electrophysiology. Additionally, induced pluripotent stem cells (iPSCs) were generated from a glaucoma patient exhibiting an E50K mutation in the Optineurin (OPTN) gene, associated with degeneration of RGCs leading to normal tension primary open angle glaucoma. These cells were differentiated to an RGC fate and assayed for phenotypic differences compared to a wild type line of iPSCs. These results will facilitate the use of hPSC-derived RGCs to study the progression of neuronal development and degeneration, the development of drug therapeutics and the use of hPSC-derived RGCs for cell replacement therapies.

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Poster

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

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Topic: A.03. Stem Cells and Reprogramming

Title: Human iPSC-derived cortical neuron model of Huntington's Disease

Authors: ***V. B. MATTIS**[1], D. RUSHTON, 90048[2], S. YOUNESI[2], C. TOM[2];
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Abstract: Induced pluripotent stem cell (iPSC) models of Huntington's disease (HD) provide an opportunity to study the mechanisms underlying disease pathology in patient tissues relevant to disease. These human-based studies are critical, due to inherent differences in cortical development and timing between humans and mice. The most prominent cell loss in HD patients occurs in cerebral cortex and striatum. In transgenic HD mice, the electrophysiological signaling has been shown to be altered even before behavioral phenotypes are present. We therefore differentiated HD and non-diseased iPSCs into functional cortical neurons by recapitulating developmental events. The HD iPSC-derived cortical neurons display key electrophysiological and immunocytochemical phenotypes as compared to their non-diseased counterparts.

Disclosures: **V.B. Mattis:** None. **D. Rushton:** None. **S. Younesi:** None. **C. Tom:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 581.14/B1

Topic: A.03. Stem Cells and Reprogramming

Support: FENS

Title: Infiltrating T lymphocytes reduce myeloid phagocytosis activity in synucleinopathy model

Authors: *A. SOMMER[1], T. FADLER[1], E. DORFMEISTER[1], A.-C. HOFFMANN[2], W. XIANG[2], B. WINNER[1], I. PROTS[1];
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Abstract: Synucleinopathies comprise a group of neurodegenerative diseases associated with abnormal accumulation of α-synuclein, among them Parkinson´s Disease (PD) is the most prominent. One of the key factors contributing to the progression of synucleinopathies is neuroinflammation. Besides activation of the innate immune cells (CNS myeloid cells), adaptive immune cells are also thought to be involved in the immune response. However, the role of the adaptive immune system, especially lymphocytes, in synucleinopathies like PD remains largely unclear. To investigate how lymphocytes impact synucleinopathies, human wild type α-synuclein (WTS) transgenic mice were crossed with mice lacking mature lymphocytes ($Rag2^{-/-}$) to answer the question. Using this *in vivo* model, we determined α-synuclein aggregation as well as numbers of the innate and adaptive immune cells in the midbrain of the central nervous system (CNS). Compared to WTS^{+} $Rag2^{+/+}$ mice, where T but not B lymphocytes infiltrated the CNS, decreased amounts of α-synuclein aggregates were found in WTS^{+} $Rag2^{-/-}$ mice lacking mature lymphocytes. Besides, the number of $Iba1^{+}$ CNS myeloid cells was not altered in presence of T lymphocytes, but we found increased frequencies of the $CD11b^{+}$ $CD45^{hi}$ population in the CNS, indicative of an increased number of infiltrated myeloid cells. Subsequently, the CNS myeloid cells were further classified according to their activation phenotype (M1 vs. M2) by gene and protein expression analysis. Interestingly, genes associated with the M1 myeloid phenotype were higher expressed in WTS^{+} $Rag2^{+/+}$ mice, whereas in the absence of lymphocytes in WTS^{+} $Rag2^{-/-}$ mice genes associated with anti-inflammatory M2 myeloid phenotype were upregulated. M1 myeloid cells are also known to perform less phagocytosis activity compared to the M2 phenotype. To proof, whether the presence of T or B lymphocytes might influence the phagocytic activity of the CNS myeloid cells, BV2 microglia were cultured with aggregated α-synuclein in presence or absence of T or B lymphocytes *in vitro.* In the presence of T but not B lymphocytes, significantly less α-synuclein was phagocytosed by BV2 microglia, further supporting the prevalence of the M1 phenotype in the presence of T lymphocytes. In conclusion, T lymphocytes strongly contribute to increased α-

synuclein pathology via modulation of the CNS myeloid cell function. In the presence of T lymphocytes, microglia phagocytosis of aggregated α-synuclein is reduced, which increases the severity of synucleinopathy.

Disclosures: **A. Sommer:** None. **T. Fadler:** None. **E. Dorfmeister:** None. **A. Hoffmann:** None. **W. Xiang:** None. **B. Winner:** None. **I. Prots:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 581.15/B2

Topic: A.03. Stem Cells and Reprogramming

Support: VCOM Internal Research Funds

Title: Creation of neural cell models of Gaucher disease type 2 from patient derived iPSC to study mechanisms of pathology.

Authors: *C. E. MCKINNEY, K. BAUMGARNER;
Genetics/Stem Cell Lab., Edward Via Col. of Osteo. Mediine, Spartanburg, SC

Abstract: More than half a century has passed since the enzymatic defect underlying Gaucher disease was appreciated. Yet, major questions regarding disease mechanisms driving pathogenesis remain. This is especially true for the neuropathic form where there are currently no effective therapies. The ability to reprogram human cells (for example; blood PBMCs and fibroblasts) to create human induced pluripotent stem cells (hiPSC) pioneered by Yamanaka has profound implications for studies of neurodegenerative disease and for applications in regenerative medicine. Here we describe neural cells derived from patient hiPSC to investigate pathologic mechanisms involved in neuronopathic Gaucher disease (GD2). This neural source material previously unavailable for study addresses the unmet, critical need to understand molecular mechanisms involved in driving neurodegeneration in Gaucher cells.
We obtained GD2 fibroblasts (Coriell Institute) where cell lines are L444P homozygotes or L444P compound heterozygotes. BJ control fibroblasts (ATCC) and control PBMCs from blood were also obtained. We created hiPSC lines from these sources by reprogramming with Yamanaka factors using a non-integrating Sendai virus carrier. Characterization of these hiPSC included mycoplasma screening, karyotyping and immunocytochemistry for pluripotent markers. *In vitro* differentiation shows these lines are capable of generating endoderm, mesoderm and ectoderm tissues. Digital droplet PCR (ddPCR) using cDNA from each GD2 and control line shows the original GD2 fibroblasts lines have decreased GBA1 (lysosomal glucocerebrosidase)

expression and a moderate increase in GBA2 (non-lysosomal glucocerebrosidase) expression when compared to controls. Using the novel GD2 and control hiPSC lines, we derived neural progenitor cells (NPCs) expressing the neuro-markers, SOX2, nestin and hSynapsin-EGFP. NPCs from each line were transformed into neurons by a defined growth medium containing supplements to drive differentiation. Endpoint PCR confirmed expression of neural biomarkers (e.g., NeuN, MAP2, β-tubulin III,) and ddPCR tracked the expression of non-lysosomal GBA2, lysosomal GBA1 and other lysosomal genes. Western blots and enzyme assays define the reduction of glucocerebrosidase presence and activity. Neural exosomes isolated from conditioned medium were characterized for exoproteins. We are beginning to investigate RNA cargo profiles in the neural exosomes. Using these neurons, we anticipate more clearly defining the pathogenesis of GD2 and creating a model to test therapeutic strategies for this inborn error of metabolism.

Disclosures: C.E. McKinney: None. **K. Baumgarner:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 581.16/B3

Topic: A.03. Stem Cells and Reprogramming

Title: Generation and characterization of retinal ganglion cells derived from human induced pluripotent stem cells

Authors: *J. C. HADERSPECK, K. ACHBERGER, S. LIEBAU;
Inst. of Neuroanatomy and Developmental Biol., Eberhard Karls Univ. Tübingen, Tübingen, Germany

Abstract: Visual impairments are a major health issue in people across all age groups and throughout the globe. Among these, retinal disorders are a leading cause for blindness, affecting mainly photoreceptors as the photosensitive layer of the eye, as well as retinal ganglion cells (RGCs) that form the optic nerve and transmit visual information from the retina to the brain. RGCs can be disrupted for a variety of different reasons, including inflammatory, traumatic, ischemic or hereditary reasons, resulting in partial or total loss of vision.Induced pluripotent stem (iPS) cells provide exciting new opportunities in the field of regenerative and personalized medicine. The capacity of iPS cells for unlimited self-renewal and their potential to differentiate into virtually every cell type of the human body has led to a steadily increasing number of protocols published that describe the molecular requirements for the respective differentiation processes. Although the human eye is a complex organ, with the retina alone consisting of at

least 5 main classes of neurons, it was shown that the development of this organ can now be recapitulated *in vitro.* Our group has successfully generated retinal organoids from human iPS cells, resembling an early developmental stage of optic vesicle-like structures and expressing markers for different retinal cell types. In addition, using a combined floating and adhesion protocol, we have generated RGCs with long axons from human iPS cells. RGC maturation and morphology are analyzed with the help of RGC-specific promoter constructs and by expression analysis of markers for RGCs at different stages. This source of RGCs can now be used to further investigate the development and maturation of the human retina and the optic nerve, as well as for disease modeling of retinal disorders. Using patient-derived somatic cells as starting material for reprogramming and subsequent differentiation into RGCs, we aim to use this model to elucidate RGC-associated disease phenotypes *in vitro*.

Disclosures: J.C. Haderspeck: None. **K. Achberger:** None. **S. Liebau:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 581.17/B4

Topic: A.03. Stem Cells and Reprogramming

Title: Establishing phenotypes in human-IPSCs for stem cell therapy after white matter stroke

Authors: *J. A. MAZZITELLI[1], I. L. LLORENTE[1], J. CINKORNPUMIN[2], W. E. LOWRY[2], S. T. CARMICHAEL[1];
[1]Neurol., [2]Molecular, Cell, Developmental Biol., UCLA, Los Angeles, CA

Abstract: Approximately one quarter of all strokes in humans occur in white matter, and the progressive nature of white matter lesions often results in severe physical and mental disability. WMS is characterized by a significant loss of axons and oligodendrocytes, as well as a migration of astrocytes to the outside of the infarct area. This specific structural disruption and cell loss suggests potential targets for WMS therapies. Two human induced pluripotent stem cell (iPSC) lines were recently introduced as appealing cell sources for cell transplantation to repair neuronal networks disrupted by ischemic stroke: iPSC-neural precursor cells (NPCs) and iPSC-glial enriched progenitor cells (GEPs). iPSCs were derived from human dermal fibroblasts through ectopic expression of the transcription factors KLF4, OCT4, SOX2, and C-MYC. NPC differentiation was induced via addition of all-trans retinoic acid to iPSCs. GEP differentiation was induced by treating NPC cultures with an iron chelator (deferoxamine), resulting in activation of hypoxia inducible factor (HIF) and enhanced gliogenesis. In order to assess the phenotype of these iPSCs and thus determine their potential for cell transplantation therapy in

WMS, iPSC-NPCs and iPSC-GEPs were cultured in the presence of growth factors and in growth factor withdrawal and then stained for known neuronal and astrocytic markers at 15 days, 2 months, and 4 months. Cells were also stained for proliferation and pluripotency markers at 15 days, 2 months, and 4 months. Immunofluorescent staining at 15 days showed increased expression of the immature neuronal marker Doublecortin in iPSC-NPCs and immature astrocytic marker GFAP in iPSC-GEPs. Proliferation and pluripotency markers (Ki67, Pax6, Sox2) also showed increased expression in iPSC-NPCs and iPSC-GEPs at 15 days. At 4 months, iPSC-NPCs and iPSC-GEPs showed increased expression of mature neuronal (NeuN) and astrocytic (S100 Beta) markers, respectively. Both cell lines showed significant downregulation of proliferation and pluripotency markers (Pax6, Sox2) after 4 months. iPSC-NPCs and iPSC-GEPs display proper differentiation and determined neuronal and astrocytic fates, respectively, indicating their potential role in phenotype-specific cell transplantation therapies in WMS.

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

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 581.18/B5

Topic: A.03. Stem Cells and Reprogramming

Support: NSF DGE-1143954

NIH Director's Innovator Award (DP2)

Presidential Early Career Award for Scientists and Engineers

Title: Modeling Huntington's disease with medium spiny neurons directly reprogrammed from patient fibroblasts

Authors: *M. B. VICTOR, M. RICHNER, A. S. YOO;
Developmental Biol., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Huntington's disease (HD) is an inherited neurological disorder that typically manifests in midlife and is characterized by two cellular hallmarks, the presence of misfolded and aggregated protein inclusions, and massive degeneration of striatal medium spiny neurons (MSNs). Human neurons derived from HD patient induced pluripotent stem cells (iPSCs) demonstrate lower resilience to cellular stressors, but do not exhibit mutant huntingtin protein aggregates. Here we show the capability of neurogenic microRNAs, miR-9/9* and miR-124, in

combination with striatum-enriched transcription factors, to efficiently convert fibroblasts from multiple patients with HD into MSNs that bear inclusion bodies (IBs) rich in amyloid-like filaments as detected by immunoelectron microscopy. Moreover, MSNs reprogrammed from HD patients also exhibit lower survivability in culture in comparison to cells reprogrammed from age- and gender-matched healthy controls. Interestingly, HD fibroblasts directly reprogrammed into cortical neurons instead of MSNs still exhibit IBs, yet with sustained survivability in culture. Our results suggest that contrary to neurons derived from induced pluripotent stem cells, directly reprogrammed neurons may be better suited for modeling late-onset diseases and that the faithful acquisition of specific neuronal-subtype identities is critical for disease modeling. Our microRNA-mediated approach to model neuronal subtype-specific vulnerabilities in HD may also prove to be advantageous in modeling other neurodegenerative disorders.

Disclosures: **M.B. Victor:** None. **M. Richner:** None. **A.S. Yoo:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 581.19/B6

Topic: A.03. Stem Cells and Reprogramming

Support: NWO Vici Grant 865.09.002

Title: Epigenetic profiling of *In vitro* generated midbrain dopamine neurons to study the role of mobile genetic elements in Parkinson's disease

Authors: N. L. HARING[1], M. P. SMIDT[1], *F. M. JACOBS[2];
[1]Swammerdam Inst. for Life Sci., [2]Swammerdam Inst. for Life Sciences, Fac. of Sci., Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: The multifactorial ethiology of Parkinson's disease (PD) and other neurodegenerative diseases implies the existence of master regulators that control the expression of gene groups involved in pathogenesis. There is an increasing body of evidence that put forward retrotransposons as potential key players in gene-regulatory pathways associated with neural development, aging, and disease. Retrotransposons are parasitic DNA sequences that shaped our genome over the course of evolution via their copy-and-paste strategy. Besides the mutagenic effects of retrotransposition events through insertion into coding sequences, mobile genetic elements can function as enhancers for nearby genes. Their activity is regulated via epigenetic repression recruited by KRAB zinc-finger proteins. Reduction of repressive histone marks and DNA methylation during aging could release the repression of retrotransposons, potentially

leading to abberant activation of genetic cascades underlying PD pathology.
In this study we profile the epigenetic landscape of human embryonic stem cell (hESC)-derived midbrain dopamine (mDA) neurons to unveil the epigenetic regulation of retrotransposons in the cell type that is specifically affected in PD. Subsequently, we model the epigenetic landscape of PD by chemically inducing DNA hypomethylation in hESC-derived mDA neurons to study the effects on retrotransposons activity and the genes in their close vicinity.
At the time of submission the study was still ongoing, results and conclusions will be presented at the poster.

Disclosures: **N.L. Haring:** None. **M.P. Smidt:** None. **F.M. Jacobs:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

Location: Halls B-H

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Program#/Poster#: 581.20/B7

Topic: A.03. Stem Cells and Reprogramming

Support: Department Funds

Title: Multiparametric measurements of the effects of neural stem cells on mild and moderate traumatic brain injuries in mice using longitudinal magnetic resonance imaging

Authors: *R. C. BOGGS[1,2], L. WATTS[2], P. FOX[2], G. CLARKE[2], M. M. DAADI[1,2];
[1]Texas Biomed. Res. Inst., San Antonio, TX; [2]Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: Traumatic brain injury (TBI) is a major cause of morbidity and mortality worldwide. In the general population, TBI poses a greater risk in athletes and military personnel making these groups particularly susceptible to TBI related ailments. Cognitive and functional deficits following TBI are often life altering and severely limits the quality of life in TBI patients. Direct mechanical damage causes neuronal and vasculature alterations that can lead to functional and structural changes in the blood vessels, gray and white matter that manifest as functional impairments like motor deficits, memory impairment, depression and anxiety. A prospect in reversing functional deficits associated with TBI is neural stem cell (NSC) transplantation, which has been used as a method of promoting brain repair by replenishing the damaged cell pool in several neurological disease models. NSCs have been shown to engraft with host tissue in animal models of brain injury and to promote plasticity and functional recovery. However, further studies are needed to bring these therapeutic approaches to the clinic. We have developed a TBI animal model for transplantation of NSCs to test neural repair of axonal injury, cerebral blood flow (CBF), reduction of lesion volumes and behavioral improvements. We used a controlled

cortical impact device to induce mild and moderate TBI in the left hemisphere of the cortex in adult CB57BL/6 mice. Magnetic resonance imaging (MRI) scans used are Diffusion Tensor Imaging (DTI) for Fractional Anisotropy (FA) and Apparent Diffusion Coefficient (ADC) maps, Arterial Spin Labeling CBF and Rapid Acquisition with Relaxation Enhancement (RARE) T_2 scans. ADC is used for early detection of ischemic brain injury and FA to evaluate damage to the white matter. RARE T_2 scans and T_2 maps can show the anatomy and size of the lesion, respectively, and CBF changes are predictive of the future progression of lesion volume. We used a battery of behavioral tests to assess motor and cognitive deficits and recovery. Behavioral data show increased anxiety, depression, and decreased motor coordination in TBI mice as seen using Light-Dark Box, Forced Swimming, Beam Walking, Cylinder, and Foot Fault Tests, respectively. MRI scans confirmed severity and location of lesion, diffusion and blood flow deficits using RARE-T_2, FA and ADC maps, and ASL scans, respectively. Our TBI model does increase anxiety and depressive behavior as compared to baseline with a reduction in motor coordination likely from hindering the right forepaw. ADC scans show a wider area of injury than T_2 while FA shows evidence of white matter damage.

Disclosures: R.C. Boggs: None. **L. Watts:** None. **P. Fox:** None. **G. Clarke:** None. **M.M. Daadi:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 581.21/B8

Topic: A.03. Stem Cells and Reprogramming

Support: Worth Family Fund, Stevens Foundation

Title: MRI guided delivery of neural stem cells into the CNS of nonhuman primates

Authors: K. MALLOY[1], G. CHOUDHURY[1], J. LI[2], A. TORRES[1], S. GUPTA[3], C. KANTORAK[3], T. GOBBLE[3], P. FOX[2], G. CLARKE[2], *M. DAADI[1];
[1]Texas Biomed, Southwest Natl. Primate Res., San Antonio, TX; [2]Univ. of Texas Hlth. Sci. Ctr. at San Antonio,, San Antonio, TX; [3]3MRI-Interventions, Irvine, CA

Abstract: Optimizing neural stem cell (NSC) delivery procedures is critical to the success of cell therapy. Unlike small molecules and growth or neurotrophic factors, cells are sensitive to their host microenvironment. Cell injection variables, such as flow rate, needle diameter, cell density, tissue porosity and mechanics, affect the penetration and dispersion of the cells into the target tissue and therefore, may compromise efficacy. In this study, we used magnetic resonance

imaging (MRI) to guide and accurately transplant NSCs into specific structures of the central nervous system (CNS). NSCs were labeled with the contrast agent Super Paramagnetic Iron Oxide (SPIO) nanoparticles, which visualizes the NSC grafts as hypointense regions on MRI. The injection parameter infusion speed and cannula diameter were initially established using a brain surrogate phantom gel model. The SPIO-labeled NSCs were injected into 0.6% agarose gel phantoms under real-time MRI to monitor the dispersion of the NSCs. The selected optimal injection parameters were used for the ClearPoint^{TM} MRI-guided delivery system into the putamen of baboon. A whole-brain 3D MRI series with ClearPoint Software established a 3D coordinate system. A cannula trajectory guide (SmartFrame^{TM}) with four MRI-sensitive fiducial markers was attached to the skull. The fiducial markers allowed the software to segment the SmartFrame^{TM} and determine the trajectory of the cannula. Iterative imaging was used to align the cannula trajectory with the target until there was less than 1 mm error between the desired target and cannula trajectory. Once the cannula trajectory was finalized, a burr hole was drilled at this location and the cannula loaded with SPIO-labeled NSCs was inserted through the guide into the brain. The injection was then initiated at 1μL/min and recorded in real-time with a TurboFlash series. A whole-brain 3D image series was acquired post-injection to visualize the graft. The post-injection MRI images confirmed that NSCs were successfully delivered to the putamen. The evidence suggests MRI guidance offers a safe, accurate and minimally invasive procedure to deliver cells to the CNS.

Disclosures: **K. Malloy:** None. **G. Choudhury:** None. **J. Li:** None. **A. Torres:** None. **S. Gupta:** None. **C. Kantorak:** None. **T. Gobble:** None. **P. Fox:** None. **G. Clarke:** None. **M. Daadi:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 581.22/B9

Topic: A.03. Stem Cells and Reprogramming

Support: Worth Fund, Stevens Foundation

Title: Differentiation and transplantation of dopaminergic neurons derived from IPSC-LRKK2 in nonhuman primate.

Authors: *G. ROY CHOUDHURY[1], A. TORRES[1], G. YANG[1], M. M. DAADI[1,2];
[1]Texas Biomed. Res. Inst., San Antonio, TX; [2]Departments of Cell. & Structural Biol., Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder caused by the death of dopaminergic neurons in the substantia nigra pars compacta (SNc). The loss of dopaminergic neurons in the SNc results in a range of debilitating movement disorders, such as bradykinesia, tremor, rigidity and postural instability in the affected patients. Pluripotent stem cells (PSCs) are specialized cells that can be differentiated into the three germ layers and serve as a valuable source for cell-based replacement therapies. Evidence indicates that stem cell derived dopaminergic neurons are a promising avenue in the search for a viable long-term treatment in PD patients. Here, we report the isolation of self-renewable neural stem cells (NSCs) from human induced pluripotent stem cells (iPSCs) derived from Parkinson's disease patients with LRRK2 (G2019S) mutation. The NSCs demonstrated the ability to proliferate in response to epidermal growth factor (EGF) and basic fibroblast growth factor (FGF2). The isolated NSCs were further differentiated in glial conditioned media (GCM) with FGF2 into neurons of dopaminergic lineage. Following differentiation, immunohistochemistry analysis revealed the presence of β-tubulin positive neurons that co-localized with the dopaminergic neuron marker tyrosine hydroxylase. Quantitative analysis of the immunohistochemistry staining demonstrated that the number of dopaminergic neurons from NSCs differentiated in GCM was significantly higher on days 1, 2 and 7 in vitro compared to control-treated NSCs. In the next step, the differentiated cells were transplanted into the putamen of a nonhuman primate (Callithrix jacchus) and the animal was monitored for a period of 1 month. The object retrieval task, with barrier detour and activity analysis, was validated as a behavioral test for evaluating motor and cognitive functions. In conclusion, we have isolated of NSCs from human iPSCs that can be differentiated into dopaminergic neurons using glial conditioned media and growth factors. Furthermore, we have successfully transplanted the differentiated dopaminergic neurons into the putamen of a nonhuman primate.

Disclosures: **G. Roy Choudhury:** None. **A. Torres:** None. **G. Yang:** None. **M.M. Daadi:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

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Topic: A.03. Stem Cells and Reprogramming

Support: The Michael J Fox Foundation

Harvard Stem Cell Institute

The Consolidated Anti-aging Foundation

Title: Dysregulation of ER stress response and calcium homeostasis in human iPSC-derived neurons carrying the LRRK2 G2019S mutation

Authors: *J. A. KORECKA[1], S. TABLOT[2], S. LEVY[1], S. M. DE LEEUW[1], M. L. TERPSTRA[1], T. M. OSBORN[1], P. J. HALLETT[1], F. M. JODELKA[3], C. J. WOOLF[2], M. L. HASTINGS[3], O. ISACSON[1];
[1]Neuroregeneration Res. Inst., McLean Hospital/Harvard Med. Sch., Belmont, MA; [2]FM Kirby Neurobio. Ctr., Children's Hosp. Boston, Boston, MA; [3]Dept. of Cell Biol. and Anat., Chicago Med. Sch. Rosalind Franklin Univ. of Med. and Sci., Chicago, IL

Abstract: The Leucine-Rich Repeat Kinase (LRRK2) G2019S gain of function gene mutation is one of the most prevalent mutations contributing to Parkinson's disease (PD) pathogenesis. The increased kinase activity alters mitochondrial health, axon outgrowth, intracellular trafficking and autophagy. We have previously shown that human LRRK2 G2019S iPS-derived neurons exhibit increased vulnerability to PD associated cell stressors and modify mitochondrial dynamics, which can be rescued by LRRK2 inhibitors (Cooper et al., 2012, Sci Transl Med. 2012, 4;4(141):141ra90.). Human iPS-derived neurons carrying LRRK2 G2019S mutation and challenged with the endoplasmic reticulum (ER) calcium (Ca^{2+}) uptake blocker thapsigargin (THP) show significantly decreased ER stress responses accompanied by neurite collapse when compared with healthy subject controls. As THP blocks ER Ca^{2+} influx via sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) and induces ER stress, this indicates that iPS neurons carrying LRRK2 G2019S mutation exhibit an altered capacity to regulate Ca^{2+} homeostasis. Indeed, we further discovered that after THP-induced SERCA block human iPS-derived neurons carrying the LRRK2 G2019S mutation exhibit an increase in depolarization-induced calcium influx in to the cell and modified calcium decay (interpreted as buffering capacity), when compared to healthy subject control neurons. This phenotype is diminished by treatment with antisense oligonucleotides targeting LRRK2 G2019S mutation. These data indicate that the LRRK2 G2019S mutation alters intracellular calcium homeostasis and ER stress response, phenotypes that could contribute to PD neuronal dysfunction.

Disclosures: J.A. Korecka: None. **S. Tablot:** None. **S. Levy:** None. **S.M. de Leeuw:** None. **M.L. Terpstra:** None. **T.M. Osborn:** None. **P.J. Hallett:** None. **F.M. Jodelka:** None. **C.J. Woolf:** None. **M.L. Hastings:** None. **O. Isacson:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

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Innovation and Technology Fund for State Key Laboratory (ITCPT/17-9)

Shenzhen Peacock Plan

SH Ho Foundation

Title: Characterization of pathogenic mutations and cellular phenotypes of Alzheimer's disease using human induced pluripotent stem cells

Authors: ***T. YE**[1,2,3], X. TU[1,2,3], E. P. TONG[1,2,3], A. K. 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: The ability to generate neurons derived from induced pluripotent stem cells (iPSCs) from normal individuals and patients promises to fill a critical gap between human and animal studies, and will provide new insight into the pathogenesis of neurological disorders such as Alzheimer's disease. Here, we established a multistep protocol to generate individual-specific cortical neurons with astrocytes in vitro from human iPSCs. First, iPSCs are directly induced into a neuronal lineage by dual inhibition of SMAD signaling. The induced cells are positive for neural progenitor cell markers nestin and Sox2. Second, the neural progenitor cells go through an extended period of neurogenesis and terminal neuronal differentiation, as evidenced by ubiquitous Tuj1- and MAP2-positive neurons. Astrocytes are generated towards the end of neurogenesis. Finally, the differentiated neurons mature, developing dendritic spines and acquiring electrophysiological properties. We are currently using this model to investigate the biochemical and synaptic dysfunction exhibited by neurons derived from healthy donors and Alzheimer's disease patients with pathogenic mutations.

Disclosures: **T. Ye:** None. **X. Tu:** None. **E.P. Tong:** None. **A.K. Fu:** None. **N.Y. Ip:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

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Program#/Poster#: 581.25/B12

Topic: A.03. Stem Cells and Reprogramming

Support: BBSRC

PSP cure

Wellcome-Trust

Title: Resolving the neuronal and glial component of tauopathies with human induced pluripotent stem cells.

Authors: *S. AGATHOU[1], M. IOVINO[2], M. SPILLANTINI[2], R. KARADOTTIR[2];
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Abstract: Frontotemporal Dementia with Parkinsonism linked to Chromosome 17 (FTDP-17T) comprises 50% of all dementia cases under the age of 60 and involves the aggregation of the microtubule-associated protein tau. Studies of tauopathies have mainly based on transgenic animals, whereby over-expression of human-specific tau is engineered. However, neurodegenerative diseases are mainly restricted to humans, and tau isoform expression differs greatly between species. Moreover, accumulating evidence indicates a contributory role for oligodendrocytes (OLs) in tauopathies. Alongside neuronal tangles, glial tangles have been observed in FTDP-17T and *in vivo* OL tau expression is sufficient to induce tauopathy. Our aim was to overcome the above limitations and to investigate the glial contribution to tauopathies by generating and characterising human neurons and OLs from induced pluripotent stem cells (iPSCs) of patients with FTDP-17T tau mutations. Whole-cell patch clamp recordings, calcium imaging and molecular biology techniques were used to examine the effects of the N279K and P301L tau mutations on membrane properties, ion-channel composition, neurotransmitter signalling and tau isoform expression in both human neurons and OLs. We demonstrated that hiPSC-derived FTDP-17T neurons recapitulate tau pathology *in vitro* and thus provide a good tool for investigating human neurodegeneration. We discovered that tau mutations accelerate neuronal maturation and increase the excitability of human iPSC-derived cortical glutamatergic neurons. Moreover, FTDP-17T human neurons have mitochondrial trafficking deficiencies and mutation-specific morphological and molecular phenotypes. We generated oligodendrocyte precursor cells from FTDP-17T and control hiPSCs, both of which matured into myelin-producing axon-interacting OLs. We showed that human and rodent OLs express tau in culture and we investigated the effect of the N279K-tau mutation on basic membrane properties, ion-channel expression and neurotransmitter signalling in human OLs. We are currently investigating the effect of mutant tau on the myelinating potential of human OLs by generating a human myelinating co-culture *in vitro*, with hiPSC-derived neurons and OLs. In summary, we showed that pathologic tau mutations affect the excitability of human neurons and that human OLs express tau although its role and the effect of tau mutations are still unclear The outcomes of this study have increased our understanding of the pathological mechanisms governing human tauopathies.

Disclosures: **S. Agathou:** None. **M. Iovino:** None. **M. Spillantini:** None. **R. Karadottir:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 581.26/B13

Topic: A.03. Stem Cells and Reprogramming

Title: C9ORF72 ALS patient motor neuron cultures exhibit protein-level dysregulation in mitochondrial cristae organization complexes

Authors: ***P. DURVASULA**, A. KAUS, B. MANDEFRO, A. GROSS, A. KERL, W. YANG, D. SAREEN;
Advanced Hlth. Sci. Pavilion, Room 8405, Cedars-Sinai Med. Ctr., Los Angeles, CA

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease affecting upper and lower motor neurons (MNs). While ALS is largely sporadic, intronic G_4C_2 repeats in *C9ORF72* (C9-ALS) have been identified in a significant subset of familial and sporadic patients. Proposed mechanisms of degeneration include oxidative stress, aberrant axonal transport, protein aggregation and bioenergetics failure. Interestingly, mitochondrial dysfunction may possess a role in many of these mechanisms.
We identified dysregulated mitochondrial proteins in multiple C9-ALS patient induced pluripotent stem cell (iPSC)-derived MN (iMN) cultures via quantitative tandem mass tag (TMT) proteomic analysis in comparison to a healthy control group. Among the 5,433 protein groups analyzed, 208 displayed significant differences in C9-ALS mutant iMN cultures, with 89 of those being mitochondrial protein groups. Bioinformatics pathway analysis revealed that aside from oxidative phosphorylation, TCA cycle, and tRNA charging pathways, imbalances in proteins critical for cristae organization and stability were a major contributor. This is particularly relevant due to the recent discovery of a missense mutation in the CHCHD10 gene, which has been implicated as a cause of familial ALS. Specifically, CHCHD10 encodes a mitochondrial protein that is enriched at cristae junctions in the intermembrane space, and has been shown to interact with several members of the mitochondrial contact site and cristae organizing system (MICOS) complex. The MICOS complex proteins are associated with the formation and maintenance of mitochondrial cristae. Together with components of the mitochondrial intermembrane space bridging complex (MIB) and the translocases of the outer (TOM) and inner (TIM) membranes, MICOS proteins are crucial for maintaining the characteristic architecture of mitochondria. As determined by our quantitative proteomics data, at

least 10 of the MICOS-MIB-TOM complex components were dramatically up-regulated in C9-ALS iMN cultures. Evidence exists, which shows that overexpression of at least one of the members of the MICOS complex leads to a considerable extension and deformation of cristae membranes and junctions. We hypothesize that disruption of mitochondrial membrane architecture and cristae junction integrity contributes to neural cell dysfunction in ALS.
In conclusion, our results suggest protein-level molecular signs of impaired mitochondrial functioning in C9-ALS patient cultures. Identifying dysregulated elements of mitochondrial biology will expand our knowledge of ALS disease onset and progression.

Disclosures: P. Durvasula: None. **A. Kaus:** None. **B. Mandefro:** None. **A. Gross:** None. **A. Kerl:** None. **W. Yang:** None. **D. Sareen:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

Location: Halls B-H

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Program#/Poster#: 581.27/B14

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant R21 NS089441

Tuberous Sclerosis Alliance Grant #332884

Human Genetics Institute of New Jersey

Finding A Cure for Epilepsy and Seizures

Title: Cellular and molecular abnormalities in neural progenitor cells derived from Tuberous Sclerosis Complex patients

Authors: *A. ZUCCO[1], V. DAL POZZO[1], A. AFINOGENOVA[1], B. CROWELL[1], M. SHELDON[2], O. DEVINSKY[3], G. D'ARCANGELO[1];
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Abstract: Tuberous sclerosis complex (TSC) is an autosomal dominant disorder caused by the mutation of either *TSC1* or *TSC2* genes. A large proportion of patients with TSC exhibit neurological symptoms including epilepsy and intellectual disability. A further 50% meet the diagnostic criteria for Autism. However, the appearance of this broad spectrum of neurologic manifestations does not always correlate with the neuroanatomical defects common to TSC,

suggesting the possibility of underlying functional defects affecting neural development of TSC patients. To investigate cellular and molecular phenotypes in TSC we generated multiple patient and sibling control-derived induced pluripotent stem cell (iPSCs) lines. Patient lines carry de novo heterozygous *TSC2* mutations. We then generated neural progenitor cells (NPCs) capable of fully differentiating into neurons, and investigated in detail their proliferation and differentiation properties. We observed that patient NPCs exhibit a consistent growth delay. Next we investigated molecular mechanisms that underlie this phenotype, and discovered specific abnormalities in the activity of the PI3K-Akt-mTOR signaling pathway that were not previously reported in heterozygous rodent models. Our findings elucidate disease mechanisms that may impact brain development and cognition in TSC.

Disclosures: A. Zucco: None. **V. Dal Pozzo:** None. **A. Afinogenova:** None. **B. Crowell:** None. **M. Sheldon:** None. **O. Devinsky:** None. **G. D'Arcangelo:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 581.28/B15

Topic: A.01. Neurogenesis and Gliogenesis

Title: Corneal Diabetes: The development of the first innervated *In vitro* model

Authors: *S. PRIYADARSINI[1], D. KARAMICHOS[2];
[1]Ophthalmology, Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK; [2]Ophthalmology & Cell Biol., Univ. of Oklahoma Hlth. Sci., Oklahoma City, OK

Abstract: Purpose: Diabetes (DM) is a leading cause of blindness in adults worldwide. Today, 284 million people are severely visually impaired worldwide, of which 39 million are completely blind. In ocular health, diabetes severely affects all ocular tissues including the cornea. To-date, studies on the diabetic cornea are limited and mainly focused around the epithelium and nerves. Several animal models have been developed however, in many occasions, the strategies and treatments that were successful in rodents failed in humans. The development of a model more relevant to the patient population is absolutely necessary. In this study we developed a 3D self-assembled corneal tissue substitute that mirrors the basic anatomical and physiological of the corneal tissue *in vivo* and can be used as a tool for investigating the defects in the human diabetic cornea as well as screening the efficacy of various agents before animal testing. **Methods:** Human corneal stromal cells from healthy (HCF), Type I (T1DM), and Type 2 (T2DM) diabetic donors. Cells were seeded on polycarbonate membrane inserts at 1million cells/well with Vitamin C stimulation to promote collagen assembly. Human corneal nerve cells (HCNs) were

obtained and seeded on top of the constructs at the 3 weeks' time point and differentiated. At week 4, all constructs were processed for RT PCR and WB. Results were analyzed using Graph Pad Prism 6. **Results:** Our study results showed upregulated nerve markers (βIII tubulin and Nestin) expression in HCNs co-cultured with T2DM (3 fold increase, $p \leq 0.001$) whereas T1DM co-cultures showed downregulated expression(1 fold, $p \leq 0.05$) for both the nerve cell markers when compared to our healthy controls. We also witnessed increased Col I (4 fold, $p \leq 0.0001$) and Col V (2.5 fold, $p \leq 0.001$) expression in T2DM which correlates with significant increase in ECM assembly and deposition. **Conclusion:** In this study we have successfully developed a novel 3D corneal substitute that helps better understanding of the crucial parameters that often gets altered during hyperglycemic conditions and are essential for the normal corneal environment maintenance. Targeting these parameters would pave the way for developing therapeutic measures in treating corneal DM complications.

Disclosures: S. Priyadarsini: None. **D. Karamichos:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 581.29/B16

Topic: A.01. Neurogenesis and Gliogenesis

Title: Integrating microRNA and mRNA expression profiles of spinal motor neuron progenitors from human embryonic stem cell

Authors: *H. CHEN[1], J. XI[4], L. HOU[2], Z. MAO[3];
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Abstract: Spinal motor neuron progenitors play a vital role in the development of motor neuron. However, the molecular mechanisms controlling this important process are largely unknown. MicroRNAs (miRs) can control mRNA expression, protein production and cell function by silencing translation or by destabilization of target mRNAs. Many studies suggested that microRNA can regulate the neural system development. In the present study, to identify the individual microRNAs (miRNAs) and mRNAs that may regulate the differentiation from human embryonic stem cell (hESCs) to spinal motor neuron progenitor, the expression profiles of motor neuron progenitors were analyzed. Microarray analyses revealed the up-regulated or down-regulated miRNAs and mRNAs in these neuronal progenitors during motor neuron development, and we found hsa-miR-9-3p, hsa-miR-551a, hsa-miR-30e-3p, hsa-miR-181d-5p, hsa-miR-200b-3p, hsa-miR-564, hsa-miR-125a-3p played important role in the motor neuron progenitor, and

also their target genes including HOXB4, HOXB9, NKX6-1, PHOX2B, TUBB3, DBX1and PTEN. These data support a crucial role for miRNAs and facilitate a potential regulatory network regulated by the genes and miRNAs during the spinal cord motor neuron differentiation.

Disclosures: H. Chen: None. **J. Xi:** None. **L. Hou:** None. **Z. Mao:** None.

Poster

581. Modeling Neurodegenerative Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 581.30/B17

Topic: A.03. Stem Cells and Reprogramming

Support: NSF RII Track 1ASSET III, #1457888

Student Undergraduate Research Fellowship from CoSM at Arkansas State University

Funds from Arkansas Biosciences Institute, Arkansas State University

Title: Extracellular environment & neural differentiation

Authors: *S. C. PANDANABOINA[1], A. ALDRIDGE[2], M. SRIVATSAN, 72401[2];
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Abstract: A constant interaction between the developing neural progenitor cells (NPC) and their immediate extracellular environment influences the process of differentiation and cell fate. Cell culture studies are crucial contributors to deciphering those interactions. Mimicking the immediate extracellular environment with strategies such as using different growth factors, scaffolds/matrices, co-cultures with other cell types for neural differentiation are approaches increasingly being used because neural cells, differentiated in significant numbers with specific phenotypes, can help transplantations in the future for functional recovery from brain/spinal cord injuries. Since vascular endothelial cells in the brain develop along with neural progenitor cells and exosomes secreted by cells are being identified as effective cell to cell communicators we tested their influence on neural differentiation. Rat NPCs (Invitrogen) were maintained in culture in Neurobasal medium with B27 supplement (Gibco) with the addition of 1% whole fetal bovine serum (FBS+Exosome) and exosome depleted FBS(FBS-Exosome). In a second set of experiments they were co-cultured with vascular endothelial cells (hCMEC/D3 cells, EMD Millipore). Immunocytochemistry using neuronal and glial markers (primary antibodies for Nestin (undifferentiated cells), NueN (for neurons) and GFAP (for astroglial cells) followed by Alexa Fluor conjugated secondary antibodies and phase contrast and fluorescence microscopy

showed that NPCs cultured for 10 days that were exposed to FBS+Exosome showed the maximum differentiation potential either into glia (13%) or neurons (29.5%) while NPCs cultured in the presence of vascular endothelial cells showed the least differentiation as 96.5% of the cells remained undifferentiated after 10 days in culture. The addition of the conditioned medium from the vascular endothelial cells still affected differentiation (89% remained undifferentiated) , however not as much as when they were co-cultured. These results suggest that exosomes in serum play an important role in differentiation while presence of vascular endothelial cells may retard differentiation. The mechanism of action of these different environments for their influence on neural differentiation is under investigation.

Disclosures: S.C. Pandanaboina: None. **A. Aldridge:** None. **M. Srivatsan:** None.

Poster

582. Modeling Psychiatric Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 582.01/B18

Topic: A.03. Stem Cells and Reprogramming

Support: CIRM TR2-01832

CIRM RB4-06277

NIH P30CA33572

Title: iPSC modeling of neurological disease

Authors: *Y. SHI[1], K. MURAI[2], G. SUN[2], E. TIAN[2], S. YANG[2], Q. CUI[2], G. SUN[2], D. TRINH[2], O. SUN[2], T. HONG[2], Z. WEN[3], M. KALKUM[2], A. RIGGS[2], H. SONG[3], G.-L. MING[3];
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Abstract: Dysregulated expression of miR-219, a brain-specific microRNA, has been observed in neurodevelopmental disorders, such as schizophrenia (SCZ). However, its role in normal mammalian neural stem cells (NSCs) and neurons and in SCZ pathogenesis remains unknown. We show here that microRNA miR-219 suppresses mouse NSC proliferation downstream of the nuclear receptor TLX. Moreover, we demonstrate up-regulation of miR-219 expression and down-regulation of TLX expression in NSCs derived from SCZ patient iPSCs and *DISC1*-mutant isogenic iPSCs. SCZ iPSC-derived NSCs exhibit reduced cell proliferation. Overexpression of TLX or inhibition of miR-219 action rescues the proliferative defect in SCZ NSCs. Moreover,

using deep sequencing analysis, we have identified a list of genes that are dysregulated in neurons derived from SCZ iPSCs and found that a set of these genes are potential miR-219 downstream targets. How elevated expression of miR-219 contributes to neuronal pathology in SCZ through regulating its downstream target genes is under investigation.

Disclosures: **Y. Shi:** None. **K. Murai:** None. **G. Sun:** None. **E. Tian:** None. **S. Yang:** None. **Q. Cui:** None. **G. Sun:** None. **D. Trinh:** None. **O. Sun:** None. **T. Hong:** None. **Z. Wen:** None. **M. Kalkum:** None. **A. Riggs:** None. **H. Song:** None. **G. Ming:** None.

Poster

582. Modeling Psychiatric Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 582.02/B19

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant U24 NS095914

Title: A human cell repository for neurological disease research

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Abstract: The National Institute of Neurological Disorders and Stroke (NINDS) Human Cell and Data Repository (NHCDR) was established in 2015 through a grant to RUCDR Infinite Biologics at Rutgers University. The NHCDR has 169 fibroblast and 72 induced pluripotent stem cell (iPSC) lines for Alzheimer's Disease (AD), Amyotrophic Lateral Sclerosis (ALS), Ataxia-telangiectasia, Frontotemporal Lobar Degeneration (FTD), Huntington's Disease (HD), Parkinson's Disease (PD), and healthy controls. These human cell lines are available to both academic and industry investigators for research purposes and can be ordered through the online NHCDR catalog (https://stemcells.nindsgenetics.org/). All iPSC lines have a certificate of analysis that summarizes the quality control assessment completed on the distribution lot. New iPSC lines will be added in 2016 and include PD iPSC lines carrying a synuclein SNCA A53T mutation, a PTEN induced putative kinase 1 PINK1 (ILE368ASN) mutation, a Parkin PARK2 (ARG275TRP) or (ARG42PRO) mutation, or a glucocerebrosidase GBA (GLU326LYS) mutation and FTD iPSC cell lines carrying a Progranulin PGRN (M1L) mutation or a Tau MAPT (P301L) mutation. The NHCDR also plans to generate isogenic lines for several of the

iPSC mutation carrying lines.
In 2016, NINDS will also add iPSC lines used by NeuroLINCS, where extensive omics data is available at www.neuorlincs.org. NeuroLINCS is one of six data and signature generating centers comprising the National Institute of Health (NIH) Library of Integrate-Network Signatures (LINCS) Common Fund program. The goal of NeuroLINCS is to establish cellular signatures for ALS and SMA, wherein the cell signatures are studied in the presence or absence of perturbants. Through a collaboration with NINDS, ten Target ALS Foundation iPSC lines can be ordered through the NHCDR catalog.
Established in 1998, RUCDR Infinite Biologics (www.rucdr.org) is the world's largest university-based integrated cell and DNA repository, assisting researchers throughout the world by providing the highest quality biomaterials, technical consultation, and logistical support. Its services include sample collection and bioprocessing (i.e., blood fractionation, nucleic-acid extraction, cell-line creation, etc.) and analytical services such as gene expression, sequencing, and genotyping. The RUCDR Infinite Biologics Stem Cell Center (RSCC) offers comprehensive services that include the reprogramming of source cells such as skin fibroblasts and blood cells to yield iPSC cells. In addition, RSCC performs a complete range of assays to characterize iPSCs to assess their quality, pluripotency and genomic stability.

Disclosures: M.L. Sutherland: None. **J.A. Tischfield:** None. **J.C. Moore:** None. **S. Saccone:** None. **M. Sheldon:** None.

Poster

582. Modeling Psychiatric Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 582.03/B20

Topic: A.03. Stem Cells and Reprogramming

Support: Governor's Council for Medical Research and Treatment of Autism CAUT13APS010

Governor's Council for Medical Research and Treatment of Autism CAUT14APL031

Title: Idiopathic autism patient-derived neural stem cells exhibit patient-specific proliferation defects in comparison to sibling control

Authors: *M. WILLIAMS[1,2], S. PREM[2], C. PINTO[3], X. ZHOU[2], P. YEUNG[2], C.-W. LUI[2], Z. PANG[2], L. BRZUSTOWICZ[4], P. MATTESON[2], J. MILLONIG[2], E. DICICCO-BLOOM[2]; [1]Neurosci., Rutgers Univ. Dept. of Cell Biol. and Neurosci., Piscataway, NJ; [2]Neurosci., Rutgers University, Robert Wood Johnson Med. Sch., Piscataway, NJ; [3]Neurosci., [4]Biol., Rutgers Univ., Piscataway, NJ

Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder defined by abnormalities in social interactions and stereotyped/restrictive behavior. Study of ASD has been hindered by disease heterogeneity and difficulties in creating representative mouse models. To define neurodevelopmental deficits in idiopathic autism, we (NJ Autism Center of Excellence; PI Millonig) generated induced pluripotent stem cells (iPSC) from 8 severely affected males and their unaffected brothers (Sib) and derived neural stem cells (NSCs). Here we focus on proliferation of ASD and Sib NSCs. To address possible variability, studies are performed blind, and all measures are assessed in multiple NSCs obtained from 2-3 independent iPSC clones for each subject. To define effects, cells were grown at high density (50K cells/cm^2) and labeled at 48h with tritiated thymidine to assess DNA synthesis and EdU for S-phase entry. In parallel, single cell analyses were conducted at 48h by acutely dissociating high-density cultures, and performing innumostaining on at low density (10K/cm^2) cultures fixed at 2h. Further, sister cultures were dissociated at 2, 4 and 6 days to quantify live cell numbers via hemocytometer. To measure protein levels, NSCs ($5x10^4$ cells/cm^2) are plated in 35 mm dishes and harvested at 48h for immunoblotting. ASD NSCs from a single-family comparison exhibit a robust and reproducible proliferation defect. In over 20 experiments utilizing NSCs derived from multiple clones per individual (2 for Sib, 3 for ASD), ASD NSCS display a 65% reduction in DNA synthesis. Additionally, ASD NSCs exhibit a 33% reduction in the proportion of cells in S-Phase as well as a 60% reduction in cell numbers after 6 days in culture. FGF, a known regulator of neurogenesis, reverses this proliferation defect in ASD NSCs by increasing cell numbers at 6 days in culture to that of Sib NSCs. Furthermore, in preliminary studies of cell cycle regulators, ASD NSCs exhibit a 65% increase in levels of cyclin-dependent kinase inhibitor p27, a negative regulator of the cell cycle. In contrast, preliminary studies of another ASD-Sib pair reveal no evidence of a proliferation defect, though the NSCs exhibit a differential response to FGF. Thus proliferation defects may be idiopathic autism patient-specific, supporting the concept of personalized medicine. In aggregate, our observations suggest we are able to discover differences in ASD-implicated biological processes and as well as potential underlying mechanisms. In comparison of an ASD-sibling pair this toolset has begun to uncover patient-specific differences in cellular phenotypes, as might be expected in idiopathic ASD.

Disclosures: **M. Williams:** None. **S. Prem:** None. **C. Pinto:** None. **X. Zhou:** None. **P. Yeung:** None. **C. Lui:** None. **Z. Pang:** None. **L. Brzustowicz:** None. **P. Matteson:** None. **J. Millonig:** None. **E. DiCicco-Bloom:** None.

Poster

582. Modeling Psychiatric Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 582.04/B21

Topic: A.03. Stem Cells and Reprogramming

Support: NINDS NS078753

Angelman Syndrome Foundation

Dup15q Alliance

Autism Speaks

NIMH MH094896

Title: Disrupted neuronal maturation in Angelman syndrome using patient-specific induced pluripotent stem cells

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

Abstract: Maternal deletion of chromosome 15q11-q13 results in a neurodevelopmental disorder known as Angelman syndrome (AS). Interestingly, individuals with a duplication of this region have a related neurodevelopmental disorder called 15q duplication syndrome (Dup15q), which in some cases is associated with autism. These syndromes are also associated with overlapping phenotypes including intellectual disability, impairments in language, and seizures. The gene believed to be responsible for AS encodes the ubiquitin ligase UBE3A. For Dup15q, it is believed that UBE3A also plays an important role, but it is likely that other genes also contribute. In both syndromes, alterations in synaptic signaling and plasticity appear to contribute to the disease phenotype, at least in mouse models, but the relevant downstream targets of UBE3A and their functional roles are unknown. Moreover, the seizure phenotype associated with these syndromes suggests the presence of an excitatory/inhibitory imbalance and/or neuronal hyperexcitability. Overall, it is clear that UBE3A is an important role-player in normal brain development and plays essential roles in excitability and synaptic function and plasticity.
In this study, we are using electrophysiological approaches and calcium imaging to examine neuronal excitability, synaptic activity, and plasticity of induced pluripotent stem cell-derived (iPSC) neurons from AS, Dup15q, and control subjects. Depolarized resting membrane potentials (RMP) were observed in both control and AS neurons early in development. Neurons derived from control subjects show a progressive hyperpolarization in RMP over development that is lacking in neurons derived from AS subjects. Similarly, control neurons show a developmental increase in spontaneous synaptic activity that is not observed in AS neurons, suggesting a genotypic difference in synapse number and/or release probability. These cellular phenotypes seem to be caused by loss of UBE3A as knockdown of this protein in control neurons and reestablishing UBE3A in AS neurons is able to mimic and rescue, respectively, these phenotypes. These iPSC-derived neurons also show spontaneous action potential firing and synchronous activity and we are currently exploring synaptic plasticity differences in these cultures using single cell recordings and multi-cell calcium imaging. Overall, these approaches may prove useful for identifying novel targets for drug discovery and for screening potential

therapeutics aimed at reversing the seizures, movement disorders, and language and cognitive impairments in AS and Dup15q.

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

Poster

582. Modeling Psychiatric Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 582.05/B22

Topic: A.03. Stem Cells and Reprogramming

Support: CT Regenerative Medicine Research Fund 13-SCCWES-01

Title: Differentiation and function of human embryonic stem cell derived GABAergic interneuron progenitors *In vitro* and in the epileptic brain

Authors: *N. C. ANDERSON[1], M. VANZANDT[1], J. GUPTA[1], S. SHRESTHA[1], C. Y. CHEN[1], D. LAWERENCE[2], G. AARON[2], J. NAEGELE[2], L. GRABEL[1];
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Abstract: The selective loss of GABAergic inhibitory interneurons is characteristic of numerous neurodegenerative diseases. Absence of these inhibitory subtypes creates an electrical imbalance in the hippocampal and cortical neural circuits. Our long-term goal is to replenish these inhibitory interneuron subtypes using an embryonic stem cell (ESC) source. During embryonic development, these inhibitory interneuron progenitors arise from a transient ventral forebrain structure known as the medial ganglionic eminence (MGE) and are characterized by the expression of Nkx2.1. We have optimized an adherent monolayer protocol for the generation of Nkx2.1+ neural progenitors from human ESCs using sonic hedgehog treatment. To test the differentiation potential of the Nkx2.1+ cells *in vitro*, we utilized co-culture and tri-culture systems with mouse cortical astrocytes and mature hippocampal cells; deriving an enriched population of interneurons in which 75% of the MAP2-positive cells are also GABA-positive after 8 weeks. Studies examining the fate of human ESC-derived ventralized neural progenitors transplanted into the mouse hippocampus of severe compromised immune deficient (SCID) epileptic mice demonstrate increased expression of the mature neuronal markers, Hu, NeuN, and the inhibitory neurotransmitter GABA between 6 and 12 weeks post transplantation. Preliminary studies suggest the transplanted cells are able to suppress recurring seizures short term in a mouse model of temporal lobe epilepsy. In addition, mice with transplanted cells exhibited significant improvement in the Morris Water Maze spatial memory task by six weeks post

transplant. Patch clamp analysis indicates that hESC derived neurons are capable of firing mature action potentials following long-term *in vitro* culture and post transplantation into mouse host.

Disclosures: **N.C. Anderson:** None. **M. VanZandt:** None. **J. Gupta:** None. **S. Shrestha:** None. **C.Y. Chen:** None. **D. Lawerence:** None. **G. Aaron:** None. **J. Naegele:** None. **L. Grabel:** None.

Poster

582. Modeling Psychiatric Disease from iPS Cells

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Topic: A.03. Stem Cells and Reprogramming

Support: NYSTEM (C026415, C026714)

NSF/CBET-1555720

Patrick P. Lee Foundation

Title: Utilizing human stem cells and cerebral organoid cultures to delineate neurodevelopmental base of schizophrenia

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Abstract: In this study we utilized neural progenitor cells (NPC) derived from schizophrenia and control iPSCs (Brennand et al., Nature, 2011) to elucidate the effects of schizophrenia on early neural development. RNA-sequencing revealed 1348 dysregulated genes common to schizophrenia patients with different genetic backgrounds accompanied by increased nuclear Fibroblast Growth Factor Receptor 1 (FGFR1)- genome interactions. Many of the dysregulated genes are involved in developmental pathways, i.e. WNT/β-Catenin and Notch signaling. The results point to an early (preneuronal) developmental-genomic etiology of schizophrenia and support the role of panontogenic Integrated Nuclear FGFR1 Signaling (INFS) in abnormal neuronal development. We developed 3D cerebral organoids from human embryonic stem cells (hESC) to analyze the role of nFGFR1 signaling in early human cortical development. The organoid rosettes recapitulated the inside-out pattern of the early stages of human cortical development. The innermost sub-ventricular-like zone (SVLZ) showed a highly dense population of proliferating KI67+ cells. The outermost molecular layer contained mature neurons and the intermediate-like zone (ILZ) contained neuroblasts and interneurons. The role of FGFR1

was examined by treating cerebral organoids with an FGFR1 antagonist, PD173074, from day 12 to day 22. PD173074 specifically depleted nuclear FGFR1 and reduced the size and the number of developing cortical rosettes (P-value< 0.001). Moreover, PD173074 decreased the density of immature neurons and interneurons in the intermediate subcortical zone, but had less effect on the earlier generated mature neurons. PD173074 did not affect the Number Area Density of proliferative Ki67+ cells, but the proliferating cells were no longer localized specifically in the SVLZ. In addition, PD173074 reduced the number of cells expressing nuclear FGFR1 in the molecular zone of cortical rosettes of cerebral organoids by 37%. Thus, blocking nuclear FGFR1 accumulation interferes with the neural development by preventing formation of the neurogenic SVZ and inhibiting neuronal differentiation. PD173074 had no effect on the Number Area Density of proliferative cells and formation of oligodendrocyte precursors. In contrast, schizophrenia iPSCs treated with PD173074 increased the size of cerebral organoids. Our results support the role of nFGFR1 in the early stage of human brain development and in its changes in schizophrenia. Genomic mechanisms of the observed developmental changes are currently being investigated by RNAseq and ChiPseq.

Disclosures: **S. Elahi:** None. **S. Narla:** None. **C.A. Benson:** None. **K. Brennand:** None. **S. Doyle:** None. **E.K. Stachowiak:** None. **M.K. Stachowiak:** None.

Poster

582. Modeling Psychiatric Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 582.07/B24

Topic: A.03. Stem Cells and Reprogramming

Support: Heinz C. Prechter Bipolar Research Fund

Richard Tam Foundation

Steven Schwartzberg Memorial Fund

Kelly Elizabeth Beld Memorial Fund

Joshua Judson Stern Foundation

Title: iPSC-derived astrocytes from bipolar disorder patients

Authors: ***C. DELONG**[1], M. BAME[2], A. WILLIAMS[2], E. MARTINEZ[1], L. AUGUSTAITIS[1], K. M. NESBITT[3], R. T. KENNEDY[4], M. MCINNIS[2], K. O'SHEA[1];

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Abstract: The directed neural differentiation of patient-derived induced pluripotent stem cells (iPSC) provides us with a developmental model to better understand the role of astrocytes in BP. Dermal fibroblasts from 4 control and 4 BP individuals were reprogrammed using episomal vectors and fully characterized for pluripotency and normal chromosome number. The iPSC lines were differentiated into neural precursors via dual SMAD inhibition, followed by a 70-day incubation in medium containing N2, fibroblast growth factor-2 (FGF-2), and epidermal growth factor (EGF) in suspension (astrospheres) followed by dissociation into astrocyte monolayer cultures. CD44-positive cells ranged from 49% to 99%, and were sorted by fluorescent activated sorting. Cell doubling times at early passage number were greater in BP lines (C 99.3$\pm$11.0, BP 130.3$\pm$31.1, p=0.054). Gene expression of aquaporin 4 and the glutamate transporter EAAT2 was significantly lower in BP lines ($P<0.05$ and $P<0.01$, respectively), and gene expression of S100beta and EAAT1 were also markedly lower in BP lines. Treatment of astrocytes with bone morphogenetic protein 4 or ciliary neurotrophic factor in the absence of FGF-2 and EGF induced differential expression of the mature astrocyte-specific marker glial fibrillary acidic protein. These differences in gene expression and behavior suggest that BP astrocytes are functionally distinct from control astrocytes. Current investigations are in progress to examine gliotransmitter release and behaviors in coculture with neurons.

Disclosures: C. Delong: None. **M. Bame:** None. **A. Williams:** None. **E. Martinez:** None. **L. Augustaitis:** None. **K.M. Nesbitt:** None. **R.T. Kennedy:** None. **M. McInnis:** None. **K. O'Shea:** None.

Poster

582. Modeling Psychiatric Disease from iPS Cells

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Program#/Poster#: 582.08/B25

Topic: A.03. Stem Cells and Reprogramming

Support: BMBF 01QE1306

Title: IPSC-derived neurons as a cellular model system for neuropsychiatric disorders

Authors: L.-M. GRUNWALD[1], M. KRIEBEL[1], M. EBERLE[2], U. KRAUSHAAR[1], F. BATTKE[3], Y. SINGH[3], A. J. FALLGATTER[2], *H. VOLKMER[1];
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Abstract: We develop iPSC-based test systems for neurodevelopmental disorders such as schizophrenia and autism. Such disorders are signified by a reduction in neuronal numbers, aberrations in neuronal outgrowth, a decrease in synaptic densities and dysfunctional molecular pathways. Based on these findings, we used iPSC-derived neurons from patients with schizophrenia or autism as a model to further elaborate morphological characteristics and to reveal basic mechanisms of underlying cognitive dysfunctions. Skin biopsies were obtained from unaffected donors and clinically diagnosed patients, the latter belonging to families with an increased case number to emphasize hereditary factors contributing to the pathology. By means of retroviral transduction isolated fibroblasts were reprogrammed into iPSCs which were characterized by immunocytochemistry and PCR. Subsequently, iPSCs were differentiated into neuronal progenitor cells and finally into neurons. Terminal neuronal differentiation was demonstrated through immunostaining for neuronal marker proteins like ?-III-Tubulin or brain layer specific proteins like CTip2. Further neurons were characterized by electrophysiological properties and transcriptome analysis. Differences in early neuronal development were assessed by neurite outgrowth measurements via high content analysis using the ImageXpress Setup. A reduced total outgrowth of neurons derived from patients with schizophrenia and autism compared to the healthy control group was revealed. The synaptogenic potential of iPSC derived neurons was determined via quantification of clusters of the postsynaptic marker PSD95. Consistently, cluster numbers were reduced in cultures of iPSC-derived neurons from patients with schizophrenia and autism, which may point towards shared disease characteristics with respect to synaptic differentiation. In conclusion, iPSC-derived neurons from patients with schizophrenia and autism showed reductions in total neurite outgrowth and a decreased formation of postsynaptic clusters, which has been also reported from post-mortem studies. These results suggest that the in vitro system described here may be a valid tool to investigate morphological and synaptic deficits associated with cognitive impairments observed in schizophrenia and autism. BMBF Fö-Kz.: 01QE1306B

Disclosures: **L. Grunwald:** None. **M. Kriebel:** None. **M. Eberle:** None. **U. Kraushaar:** None. **F. Battke:** A. Employment/Salary (full or part-time): CeGaT GmbH. **Y. Singh:** A. Employment/Salary (full or part-time): CeGaT GmbH. **A.J. Fallgatter:** None. **H. Volkmer:** None.

Poster

582. Modeling Psychiatric Disease from iPS Cells

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Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant NS083009

UCI CIRM Postdoctoral Fellowship TG2-01152

NIH Grant NS032387

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Title: Modeling epilepsy with isogenic human iPSC-derived neurons

Authors: *Y. XIE[1], N. N. NG[1], O. N. SAFRINA[1], S. E. KONOPELSKI[1], R. J. SCHUTTE[1], P. FIGUEROA[1], S. S. SCHUTTE[1], A. E. STOVER[3], K. ESS[4], A. L. GEORGE, Jr.[5], M. A. SMITH[2], P. H. SCHWARTZ[3], D. K. O'DOWD[1];
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Abstract: A large number of distinct missense mutations in the *SCN1A* gene*,* encoding $Na_v1.1$ voltage-gated sodium channels result in genetic epilepsy with febrile seizures plus (GEFS+). However, the underlying cellular mechanisms contributing to this class of seizure disorders are not well understood. Previously we explored the cellular changes in a *Drosophila* knock-in fly line carrying the GEFS+ K1270T *SCN1A* mutation. Our data indicate that this mutation contributes to hyperthermia-induced seizures through a conditional gain-of-function alteration in sodium channels that reduces excitability of GABAergic neurons (Sun *et al*., 2012). To determine whether this mutation causes similar changes in human neurons, we generated induced pluripotent stem cell (iPSC) lines from two siblings, one with the K1270T mutation (GEFS+ sibling) and one without (Control sibling). The iPSC lines were differentiated into neurons. Three weeks after plating onto an astrocyte feeder layer, the majority of cells with neuronal morphology, in both the GEFS+ and Control sibling lines, fired action potentials and received glutamatergic and/or GABAergic synaptic input. We have also used the CRISPR-Cas9 system to introduce the K1270T mutation into the Control sibling iPSC line, creating an isogenic mutant line. We are now conducting comparisons at elevated temperature to determine if there are differences in excitability and synaptic transmission between neurons differentiated from the GEFS+ sibling, Control sibling and isogenic mutant lines. These studies will provide insight into cellular mechanisms contributing to febrile seizures in *SCN1A* associated epilepsy disorders.

Disclosures: Y. Xie: None. **N.N. Ng:** None. **O.N. Safrina:** None. **S.E. Konopelski:** None. **R.J. Schutte:** None. **P. Figueroa:** None. **S.S. Schutte:** None. **A.E. Stover:** None. **K. Ess:** None. **A.L. George:** None. **M.A. Smith:** None. **P.H. Schwartz:** None. **D.K. O'Dowd:** None.

Poster

582. Modeling Psychiatric Disease from iPS Cells

Location: Halls B-H

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Program#/Poster#: 582.10/C1

Topic: A.03. Stem Cells and Reprogramming

Support: NIMH Grant R01MH099578

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Novo Nordisk Foundation

Title: Human iPSC glial mouse chimeras reveal glial contributions to schizophrenia

Authors: *K. L. MCCOY[1], S. WANG[1], L. ZOU[1], S. SCHANZ[1], J. MUNIR[1], J. BATES[1], D. CHANDLER-MILITELLO[1], Z. LIU[1], R. FINDLING[2], R. H. MILLER[3], M. NEDERGAARD[1,4], P. TESAR[5], M. S. WINDREM[1], S. A. GOLDMAN[1,4];
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Abstract: Recent genetic and neuroradiological studies have suggested a role for glial pathology in the genesis of schizophrenia. To assess this possibility, we established human glial chimeric mice using glial progenitor cells (GPCs) produced from induced pluripotential cells (hiPSCs), which were derived from patients with juvenile-onset schizophrenia and their gender- and ethnicity-matched controls. To this end, hiPSC GPCs were neonatally implanted into either immunodeficient myelin wild-type mice, in which the donor GPCs remained as progenitors or became astrocytes, or into hypomyelinated shiverer mice, in which the GPCs also gave rise to oligodendrocytes. When implanted into shiverer mice, by 4 months of age the schizophrenia-derived (SCZ) iPSC GPCs exhibited less white matter engraftment than control iPSC GPCs, instead migrating prematurely into the cortex. The density of SCZ-derived GPCs in the corpus callosum was significantly less than that in control GPC-engrafted mice (N=13; P<0.0001), which resulted in hypomyelination of the callosum (MBP expression by luminance; P< 0.0001). Moreover, the density of transferrin-defined oligodendrocytes was lower in SCZ callosum than in control-engrafted (SCZ: 15,993 ± 2,693/mm^3, N=12; control: 9,084 ± 1,249 N=16; P=0.03). Strikingly, callosal GFAP-defined astrocytic differentiation was almost 6 times higher in control-engrafted brains than in SCZ GPC chimeras (control: 6,616 ± 672/mm^3, N=12; SCZ: 1,177 ± 277/mm^3, N=19; P<0.0001). In myelin wild-type hosts, the SCZ hiPSC glial chimeras exhibited

an aberrant behavioral phenotype, characterized by: 1) diminished prepulse inhibition; 2) higher anxiety as assessed by elevated plus maze; 3) increased social avoidance in 3-chamber social testing; 4) impaired novel object recognition; and 5) anhedonia, as reflected in diminished sucrose preference, all as compared to control GPC-engrafted chimeras. Lines derived from 3 different patients and 3 age- and gender-appropriate controls were used in all experiments. These data suggest a potent contribution of cell-autonomous glial pathology to the development of schizophrenia.

Disclosures: **K.L. McCoy:** None. **S. Wang:** None. **L. Zou:** None. **S. Schanz:** None. **J. Munir:** None. **J. Bates:** None. **D. Chandler-Militello:** None. **Z. Liu:** None. **R. Findling:** None. **R.H. Miller:** None. **M. Nedergaard:** None. **P. Tesar:** None. **M.S. Windrem:** None. **S.A. Goldman:** None.

Poster

582. Modeling Psychiatric Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 582.11/DP01 (Dynamic Poster)

Topic: A.03. Stem Cells and Reprogramming

Support: Alzheimerfonden AF-556051

Title: Synchronous oscillatory activity in human iPSC derived cortical circuits

Authors: *S. ILLES, J. ISZÁK, D. VIZLIN HODZIC, Q. ZHAI, J. STRANDBERG, T. OLSSON BONTELL, E. HANSE, K. FUNA;
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Abstract: The hallmark of the brain is the ability to generate oscillatory activity categorized in different frequency bands. This required the full neuronal maturation of neural stem cell-derived neuronal progeny into structured three-dimensional (3D) organized neuronal circuits as given e. g. in cortical brain region. We assessed *in vitro* generated human induced pluripotent stem cells (hiPSC)-derived 3D-cortical neural assemblies by super-confocal-laser microscopy imaging, patch-clamp and multi-electrode array technology and present here the structural and functional properties at the synapse towards the neuronal network level. In detail, our differentiation procedure applied on hIPSC gives rise to 3D-cortical aggregates comprising early-, middle- and late-born cortical glutamatergic as well as GABAergic neurons. These neurons are organized as polarized cell-layer embedded into a glial network generated by 3D-growing mature GFAP and S100-positive astrocytes. Neurons generating a neurite net which are interconnected by vGlut1 and PSD-95 mature synapse, allowing excitatory and inhibitory neurotransmission revealed by

whole-cell recordings. Cell-attached and extracellular recordings demonstrated that neurons within hiPSC-derived cortical aggregates show mature electrophysiological properties, i.e. generation of spontaneous action potentials and bursting. Moreover, multi-electrode array recordings reveal the autonomous generation, i. e. in the absence of electrical or chemical stimulation, of synchronized activity recorded as population bursting and oscillatory local field potentials. In this poster we provide a detailed description of the properties of oscillatory LFP recorded in *in vitro* generated human cortical circuits. Here we not only demonstrating that hiPSC-derived neurons mature into functional circuits within few weeks starting from iPSC-stage, but also show that hiPSC-derived neural circuits show principles of human brain functions.

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Poster

582. Modeling Psychiatric Disease from iPS Cells

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Program#/Poster#: 582.12/C2

Topic: A.03. Stem Cells and Reprogramming

Support: Harvard Stem Cell Institute grant funds

Michael J Fox Foundation

Consolidated Anti-Aging Foundation

Title: *In vitro* and *In vivo* characterization of frozen-thawed dopaminergic cell preparations for Parkinson's disease autologous cell replacement therapy

Authors: *T. M. OSBORN[1], M. BADI[1], D. DINESH[1], J. PRUSZAK[2], A. ASTRADSSON[1], J. A. KORECKA[1], R. SPEALMAN[1], J. SCHUMACHER[1], P. HALLETT[1], O. ISACSON[1]; [1]Neuroregeneration Res. Inst., McLean Hospital/Harvard Med. Sch., Belmont, MA; [2]Inst. for Cell and Gene Therapy, Univ. Med. Ctr. Freiburg, Freiburg, Germany

Abstract: Patients with Parkinson’s disease (PD) present with motor symptoms characterized by tremor, bradykinesia, rigidity and postural instability. There are approximately 1.5 million diagnosed cases of this chronic progressive disorder in the U.S. At the onset of symptoms and diagnosis ~ 70% of the midbrain DA neurons have degenerated. L-DOPA can initially restore dopaminergic (DA) levels and motor function, but with time the therapeutic window becomes increasingly narrow with L-DOPA induced dyskinesia as a common side effect. Although deep-

brain-stimulation (DBS) also can alleviate motor symptoms, such interventions ultimately lead to repeat procedures, limitations for patients in receiving other medical procedures and high medical costs. The concept of cell replacement therapy has shown benefit in clinical studies using cell preparations derived from fetal ventral midbrain. However, fetal cell transplantations are not scalable for a larger patient population and require immunosuppression. Induced pluripotent stem cells (iPSCs) can be generated from affected PD patients, differentiated into midbrain dopaminergic cells using xeno-free procedures, and frozen-thawed for use in autologous transplantations. The proof-of-concept in non-human primates has previously been shown by us (Hallett et al. Cell Stem Cell. 2015 Mar 5;16(3):269-74). In recent pre-clinical efforts, we have differentiated episomal xeno-free iPSCs, derived from human PBMCs, into midbrain DA neurons. The cell-preparations have been frozen and thawed with reliable reproducibility. Stability, cell marker characteristics and functionality of the frozen-thawed cells and such preparations are now tested in vitro and in primate and rodent models in vivo.

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Poster

582. Modeling Psychiatric Disease from iPS Cells

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Program#/Poster#: 582.13/C3

Topic: A.03. Stem Cells and Reprogramming

Support: FA8650-13-2-6453 Department of the Air Force

Title: Dynamics of human neuronal network microcircuitry on high-throughput multielectrode arrays and application in drug screening

Authors: *S. HINCKLEY, S. BIESMANS, A. BANG;
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Abstract: Neural network activity represents the integration of multiple synaptic events and the sum output of neuronal ensembles. While human neurophysiology has been described at the single synapse level and the population field potential level of EEG, microcircuitry dynamics have not been well established. Development of technology platforms to study microcircuitry will better elucidate disease etiology and drug influence on network function in human models. Efforts toward application of higher relevance model systems in drug discovery benefit from the scalability, reproducibility and genetic background provided by patient derived induced

pluripotent stem cell (iPSC) neurons. These *in vitro* human iPSC based neural networks can be utilized for screening drugs that modulate network behavior and also for pharmacogenomic studies of human neurological disease. Using hiPSC-derived neurons, we have developed a physiological assay on multi-well multielectrode array (MEA) plates, where we simultaneously record extracellular potentials for 16 channels per well across 48-well plates. An advantage of this system, versus traditional electrophysiology, is the ability to non-invasively measure neuronal activity over days and months, utilizing each well as its own control, thereby expanding the repertoire of drug exposure paradigms to more closely resemble clinical use. hiPSC-derived neurons formed *de novo* networks on the MEA plate as evidenced by the appearance of complex synaptic activity including synchronized network bursts. Our assay displayed highly consistent well-to-well activity level and network behavior. Furthermore, manipulation of excitatory and inhibitory neurotransmission showed physiologically relevant network formation. Analysis of network function in response to a focused library of pharmacological and neurotoxin compounds revealed dynamic plasticity of network output. Development of technology platforms that can be used to perform drug screens against models of human brain microcircuitry *in vitro* with relatively high-throughput will be essential to realize their potential for disease modeling and drug discovery.

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Poster

582. Modeling Psychiatric Disease from iPS Cells

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Support: NIH Grants R01MH104701 and R01MH099578

The Leila Y. and Harold G. Mathers Charitable Foundation

The Novo Nordisk Foundation

Title: Transcriptomic analysis identifies dysregulation of glial differentiation in schizophrenia-derived oligodendrocyte progenitor cells

Authors: ***M. OSIPOVITCH**[1], Z. LIU[2], J. BATES[2], D. CHANDLER-MILITELLO[2], R. FINDLING[3], M. WINDREM[2], S. WANG[2], P. TESAR[4], S. GOLDMAN[1];
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Child and Adolescent Psychiatry, Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [4]Dept. of Genet., Case Western Reserve Univ., Cleveland, OH

Abstract: A number of studies have suggested significant glial involvement in schizophrenia. Nonetheless, the contribution of cell-autonomous glial transcriptional dysregulation to the pathogenesis of schizophrenia has not been specifically explored. To this end, we have generated mRNA sequencing data from oligodendocyte progenitor cells (OPCs) produced from human induced pluripotent stem cells obtained from fibroblasts of patients with juvenile-onset schizophrenia, as well as from age- and gender-matched healthy controls. The OPCs were prepared using previously described methods (Wang et al., Cell Stem Cell, 2013), and then enriched to near-purity using CD140a/PDGFRa-targeted fluorescence-activated cell sorting. Differential gene expression analysis (corrected P Value < 0.05 and linear fold change > 2.00) of 4 schizophrenic- and 3 control-derived cell lines revealed marked differences in differentiation- and myelination-associated genes in schizophrenic OPCs, as compared to their controls. Both differential gene expression and differential network analyses identified disruptions in the OPC differentiation signaling pathway as indicated by significant down-regulation of a coherent set of key OPC lineage transcription factors NKX2-2, OLIG1, OLIG2, SOX10, SIRT2, MYRF, and ZNF488 as well as genes involved in myelination such as MBP, PLP1, MAG, UGT8, MOG, and FA2H suggesting a relative arrest in glial differentiation at the point of OPC specification from neural stem cells. Functional analysis of schizophrenia-dysregulated genes identified synaptic transmission, differentiation of oligodendrocytes, and myelination among the most differentially affected central nervous system functions; all were significantly down-regulated. Together, these findings reveal a major role for cell-autonomous glial pathology in the etiology of child-onset schizophrenia.

Disclosures: M. Osipovitch: None. **Z. Liu:** None. **J. Bates:** None. **D. Chandler-Militello:** None. **R. Findling:** None. **M. Windrem:** None. **S. Wang:** None. **P. Tesar:** None. **S. Goldman:** None.

Poster

582. Modeling Psychiatric Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 582.15/C5

Topic: A.03. Stem Cells and Reprogramming

Title: Neuronal calcium signaling in stem cell models of bipolar disorder

Authors: *A. J. WILLIAMS[1], C. DELONG[2], M. BAME[2], E. MARTINEZ[2], R. DOUCETTE[2], R. PARENT[2], K. GLANOWSKA[2], M. MCINNIS[2], E. STUENKEL[2], G. MURPHY[2], K.

O'SHEA[2];
[1]Psychiatry, [2]Univ. of Michigan, Ann Arbor, MI

Abstract: A major challenge in understanding human neuropsychiatric disorders has been the lack of viable cells and tissues for analysis. Patient-derived induced pluripotent stem cells (iPSC) offer the opportunity to examine the full complement of neural tissues and the prospect of identifying underlying disease mechanisms. To study bipolar disorder (BP), we have derived and characterized iPSC from fibroblasts obtained from controls (C) and patients with BP, and have differentiated them into neurons and glia. With the fluorescent calcium indicator, Fluo-4 AM, we can measure differences in spontaneous and evoked neuronal responses in BP versus C neurons. While both BP and C neurons respond to depolarization, we find that BP neurons have greater calcium transients in response to certain types of stimulation than C neurons. We have also found that lithium pre-treatment reduces BP neuron calcium transients and wave amplitude to levels comparable to C neurons. We are currently using these cell models to investigate the role of rs1006737, a single nucleotide polymorphism in the *CACNA1C* calcium channel gene - and a genetic risk factor for BP - in neuronal differentiation and function. We used the CRISPR/Cas9 genome editing system to edit BP cells with the rs1006737 risk genotype (AA) into the nonrisk (GG) genotype, and we are now assessing calcium signaling and differentiation potential in the corrected cells. One potential BP therapeutic, ketamine, may act by altering calcium signals in target cells. To evaluate this, we are using our cell models to assess how ketamine alters patterning and differentiation in BP and C neurons. We are also studying mouse neurons lacking specific calcium channels to investigate how these channels mediate neuronal calcium transients observed by Fluo-4 AM. The overarching goal of our research is to identify novel disease phenotypes and mechanisms involved in bipolar disorder, with the ultimate aim of improving treatment.

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Poster

582. Modeling Psychiatric Disease from iPS Cells

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Support: Defense Threat Reduction Agency - JointScience and Technology Office, Medical S & T Division (CBM.THRTOX.01.10.RC.021)

NIAID (AOD12058-0001-0000)

Title: Functional synaptogenesis in human stem cell derived neurons for neurotoxicity studies

Authors: *M. J. STENSLIK, P. H. BESKE, K. M. HOFFMAN, K. L. SCHULZ, M. R. EISEN, D. L. NGUYEN, P. M. MCNUTT;
US Army Med. Res. Inst. of Chem. Def, Edgewood, MD

Abstract: Neurons derived from human pluripotent stem cells (hPSCs) have the potential to provide a physiologically relevant model to study the clinical manifestations of various toxins, such as botulinum neurotoxins. Numerous publications describe the differentiation of human pluripotent-derived neural stem cells (hNSCs) *in vitro,* and demonstrate morphological markers of neurotypic identity months after derivation. However, neuronal models often fail to exhibit active synapses with network-level responses. Therefore, we sought to accelerate conditions for the functional maturation of hPSCs into networked cultures of hNSCs. We have previously described the morphological and functional maturation of various hNSCs by immunocytochemistry and whole-cell patch-clamp electrophysiology. Results demonstrate that within 7 weeks of differentiation hNSCs displayed appropriate compartmentalization of post-mitotic morphological markers (e.g., MAP2, Tau and/or NeuN) as well as miniature post-synaptic currents (mPSCs). mPSCs were blocked with the treatment of CNQX, an AMPA agonist confirming excitatory AMPA receptor-mediated events. Addition of botulinum neurotoxin serotypes A or B to hNSCs resulted in SNARE protein cleavage and termination of spontaneous synaptic activity. Currently, we are investigating the maturation of human pluripotent stem cell-derived motor neurons (hPSC-PMNs). Preliminary studies indicate the presence of post-mitotic morphological neurotypic markers (e.g. MAP2, Tau and/or NeuN), and the emergence of immature neuronal activity in as early as 21 d after plating. In combination with previous findings, these data suggest that careful differentiation of commercially available human-induced pluripotent stem cell-derived neuronal populations can produce physiologically relevant, synaptically active neuronal cultures that are suitable for mechanistic and high-throughput therapeutic screening studies of biological and chemical neurotoxins.

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Poster

582. Modeling Psychiatric Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 582.17/C7

Topic: A.03. Stem Cells and Reprogramming

Support: KAKENHI, 16J02472

Title: Long-term potentiation and depression phenomena in human induced pluripotent stem cell-derived cortical neurons

Authors: *A. ODAWARA[1,2,3], N. MATSUDA[1], G. CHENG[4], R. ARANT[4], I. SUZUKI[1]; [1]Tohoku Inst. of Technol., Sendai, Japan; [2]Tohoku university, Sendai, Japan; [3]JST, Tokyo, Japan; [4]Alpha med scientific, Osaka, Japan

Abstract: Long-term potentiation (LTP) and long-term depression (LTD) in neuronal networks has been analyzed using in vitro and in vivo techniques in simple animals to understand learning, memory, and development in brain function. Human induced pluripotent stem cell (hiPSC)-derived neurons may be effectively used for understanding the plasticity mechanism in human neuronal networks, thereby elucidating disease mechanisms and drug discoveries. In this study, we attempted the induction of LTP and LTD phenomena in a cultured hiPSC-derived cerebral cortical neuronal network using multi-electrode array (MEA) systems. High-frequency stimulation (HFS) produced a potentiated and depressed transmission in a neuronal circuit for 1 h in the evoked responses by test stimulus. The cross-correlation of responses revealed that spike patterns with specific timing were generated during LTP induction and disappeared during LTD induction and that the hiPSC-derived cortical neuronal network has the potential to repeatedly express the spike pattern with a precise timing change within 0.5 ms. We also detected the phenomenon for late-phase LTP (L-LTP) like plasticity and the effects for synchronized burst firing (SBF) in spontaneous firings by HFS. In conclusion, we detected the LTP and LTD phenomena in a hiPSC-derived neuronal network as the change of spike pattern. The studies of plasticity using hiPSC-derived neurons and a MEA system may be beneficial for clarifying the functions of human neuronal circuits and for applying to drug screening.

Disclosures: **A. Odawara:** None. **N. Matsuda:** None. **G. Cheng:** None. **R. Arant:** None. **I. Suzuki:** None.

Poster

582. Modeling Psychiatric Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 582.18/C8

Topic: A.03. Stem Cells and Reprogramming

Support: Institutional seed grant

Title: Development of an *In vitro* patient specific neurovascular unit based on induced pluripotent stem cells

Authors: *R. PATEL, S. PAGE, A. ALAHMAD;
Pharmaceut. Sci., Texastech Univ. Hlth. Sci. Ctr., Amarillo, TX

Abstract: The blood-brain barrier (BBB) constitutes a component of the neurovascular unit formed by specialized brain endothelial cells (BMECs) surrounded by astrocytes, pericytes and neurons. It plays an important role in the maintenance of the brain homeostasis by the presence of a dual barrier providing a physical (tight junctions) and chemical (efflux pumps) barrier against pathogens and toxic compounds.
Dysfunction of the BBB function is associated with many neurological diseases, in particular with neurodegenerative diseases. However, current in vitro models of the human BBB are limited by their poor barrier properties, whereas the cellular mechanisms described in rodent-based models remain to be validated. In this study, we developed a patient-specific model of the BBB using patient-derived induced pluripotent stem cells (iPSCs) and differentiated these cells into BMECs, neurons and astrocytes using established protocols.
In our hands, iPSC-derived BMECs showed barrier properties similar or better than hCMEC/D3 monolayers (an immortalized human brain endothelial cell line), as they displayed the phenotype of a mature BBB.
Furthermore, we were capable to differentiate neurons and astrocytes from the same iPSCs. We demonstrated that such neurons were sensible to known neurotoxic compounds. In addition, such neurons and astrocytes were capable to up-regulate the barrier tightness in iPSC-derived BMECs monolayers upon co-cultures.
In this study, we demonstrated the ability to develop an all-inclusive and functional in vitro model of the neurovascular unit capable to integrate astrocytes, BMECs and neurons.
Our future direction is focused on using such model to better understand clearance of Abeta peptides across the BBB, as well as the impact of genetic factors on the BBB by differentiating iPSCs from patient suffering from a early onset of familial Alzheimer disease (eFAD).

Disclosures: **R. Patel:** None. **S. Page:** None. **A. Alahmad:** None.

Poster

582. Modeling Psychiatric Disease from iPS Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 582.19/C9

Topic: A.03. Stem Cells and Reprogramming

Support: Doctoral Programme in Biomedicine and Biotechnology

Finnish Cultural Foundation

Finnish Funding Agency for Technology and Innovation

Title: Effect of different human recombinant laminin isoforms on human pluripotent stem cell -derived neurons *In vitro*

Authors: *A. HYYSALO, M. RISTOLA, M. MÄKINEN, S. NARKILAHTI;
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Abstract: Extracellular matrix plays a crucial role in the central nervous system (CNS). Laminins are one of the major protein groups in the extracellular matrix participating in many aspects of the CNS physiology. These large heterotrimeric glycoproteins consist of α, β and γ chains, which can be assembled into variety of different isoforms. Biological roles of laminin isoforms in developing human CNS have not previously been extensively studied, but recently human recombinant laminin isoforms have become commercially available facilitating the research. Human pluripotent stem cells (hPSCs) can be utilized for efficient production of human neuronal cells *in vitro*. Combination of human-derived cells with defined laminin isoforms enables more detailed *in vitro* research aiming to better understand the role of different laminin isoforms in the development, function and repair of the human CNS.
The aim of this study was to compare how different human recombinant laminin isoforms support the growth and development of hPSC -derived neurons *in vitro*. The functionality of developing human neuronal networks on different laminin isoforms was studied, and to our knowledge this aspect has not been considered in any previous studies.
Commercially available, human recombinant laminin isoforms 211, 332, 411, 511, 521 (Biolamina) and human recombinant laminin 511 fragment E8 (Takara Bio Inc.) were used as cell culture substrates. Cell attachment, viability, proliferation, and neuronal differentiation were studied on different laminin isoforms using immunocytochemistry and quantitative image analyses. In addition, gene expression profiles of extracellular matrix associated molecules were compared using quantitative RT-PCR and electrophysiological measurements using microelectrode array were performed to monitor the functionality of cultured neuronal networks. Laminin isoforms containing α5-chain were most supportive for hPSC -derived neurons in terms of cell attachment, viability, proliferation, and activity development. Cells formed spontaneously active neuronal networks on all tested laminin isoforms, however, distribution of the network activity was affected by laminin isoforms. Furthermore, human recombinant laminin 511 fragment E8 alone supported growth and functional development of hPSC -derived neurons equally well compared to full length laminin molecule.
In conclusion, human recombinant laminin isoforms and specific laminin fragments can be used for efficient xeno-free and defined culturing of hPSC-derived neurons. This *in vitro* system facilitate defined research concerning laminin isoforms in human CNS.

Disclosures: **A. Hyysalo:** None. **M. Ristola:** None. **M. Mäkinen:** None. **S. Narkilahti:** None.

Poster

582. Modeling Psychiatric Disease from iPS Cells

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 582.20/C10

Topic: A.03. Stem Cells and Reprogramming

Support: Luis Sklarow Memorial Trust

Community Foundation for Greater Buffalo

Title: iPSC lines as a model system to study the dosage effect of a human specific fusion gene CHRFAM7A

Authors: *I. IHNATOVYCH, K. E. SWIECK, A. LEW, K. SZIGETI;
Neurol., SUNY At Buffalo, Buffalo, NY

Abstract: Emerging concepts point to a role for human-specific genes in complex human diseases. *CHRFAM7A* is a human specific fusion gene, a result of multiple chromosomal rearrangements on chromosome 15 during evolution. The region has ongoing instability, and various neurological phenotypes have been reported in the context of deletions and duplications. *CHRFAM7A* has been implicated in neuropsychiatric and neurodegenerative disorders; human specific diseases affecting higher cognitive function. Recently two independent CNV GWAS studies reported an association between *CHRFAM7A* dosage and Alzheimer's disease (AD). The *CHRFAM7A* fusion gene harbors a part of the alpha 7 nicotinic acetylcholine receptor (*CHRNA7*) and 4 new exons of the FAM7 sequence corresponding to a part-functional CHRNA7 and a part-kinase (FAM/ULK4) sequence. In vitro CHRFAM7A affects cell response to the canonical CHRNA7 ion-gated channel. Furthermore, it has an important role in inflammation. We report the characterization of two iPSC lines - UB019 and UB068 - with two and zero copies of CHRFAM7A, respectively. iPSCs (three colonies/line) were generated from human skin biopsies from patients with AD by episomal transformation containing Oct4, Sox2, Klf4, c-Myc and Nanog. Three colonies per line/individual were characterized for: i) chromosomal abnormalities (aCGH), ii) genome-wide DNA methylation (RRBS), iii) gene expression profiles (Affymetrix gene expression array), and iv) lineage preference using non-directed EB differentiation followed by TagMan hPSC Scorecard Panel. The colony with the highest preference for neuroectodermal differentiation was selected for the further neuronal and microglial differentiation. The iPSC lines expressed pluripotency markers by qPCR and ICC. There were no chromosomal aberrations detected in the iPSC lines. During propagation of the cell lines the *CHRFAM7A* CNV remained stable. Genome-wide DNA methylation changes were detected as expected, but did not affect *CHRFAM7A* expression levels. Non-directed differentiation confirmed the presence of markers of all three germ layers. 60% of the colonies for each line showed neuroectodermal preference of differentiation. iPSCs differentiated into

type- specific neurons or microglia provide an in vitro assay system to study the effect of a given CNV on the pathomechanism of disease. After establishing readout and rescue, this in vitro system is also adaptable for high throughput drug screen in a genotype and mechanism-specific manner.

Disclosures: I. Ihnatovych: None. **K.E. Swieck:** None. **A. Lew:** None. **K. Szigeti:** None.

Poster

582. Modeling Psychiatric Disease from iPS Cells

Location: Halls B-H

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Program#/Poster#: 582.21/C11

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant NS083688

Takeda Grant

Hartwell Fellowship MSN188293

Title: An isogenic blood-brain barrier comprising brain endothelial cells, astrocytes and neurons derived from human induced pluripotent stem cells

Authors: *S. G. CANFIELD[1], M. J. STEBBINS[1], B. S. MORALES[1], S. W. ASAI[1,2], G. D. VATINE[2], C. N. SVENDSEN[2], S. P. PALECEK[1], E. V. SHUSTA[1];
[1]Chem. and Biol. Engin., Univ. of Wisconsin Madison, Madison, WI; [2]Board of Governors Regenerative Med. Inst., Cedars-Sinai Med. Ctr., Los Angeles, CA

Abstract: The Blood Brain Barrier (BBB) is critical in maintaining a physical, metabolic, transport barrier between the blood and the brain. The BBB consists of brain microvascular endothelial cells (BMECs) that form the brain micro-vasculature and are supported by astrocytes, neurons, pericytes, and neural stem cells, that altogether form the neurovascular unit. A number of in vitro BBB models have been developed to elucidate the role of BBB in brain development, function, and disease, and to develop potential therapeutic approaches. Primary isolated or transformed BMECs dedifferentiate and lose their barrier properties once they are removed from their microenvironment and often exhibit sub-par BBB phenotypes. Inter-species variations limit the translation of a number of BBB studies to a human *in vivo* setting. Additionally, previous BBB models consist of cell types from multiple species and with primary cells isolated from animals of various developmental stages. Thus, the ability to derive stem cells from healthy and diseased human patients, differentiate them into the multiple cell types of the neurovascular unit, and investigate BBB characteristics all while maintaining the original host genotype makes a

human stem cell-derived BBB model potentially powerful for dissecting human diseases. To this end, we have successfully differentiated BMECs, astrocytes, and neurons from human induced pluripotent stem cells (iPSCs). Compared to other in vitro BBB models, these iPSC-derived BMECS exhibit more physiologic BBB phenotypes, such as a high transendothelial electrical resistance, low permeability, polarized efflux transport, and expression of tight junction markers. The addition of co-culture with neurons and astrocytes further elevated trans-endothelial electrical resistance (TEER), reduced permeability, and improved tight junction continuity in the endothelial cell population. Varying the ratio of neurons and astrocytes to match that roughly similar to the human brain (1 neuron: 3 astrocytes) was found to be the most BBB enhancing. For the first time, we have developed a completely human induced pluripotent stem cell-based BBB model. The ability to derive an isogenic BBB model consisting of astrocytes, neurons, and BMECs from the same original stem cell source could have major implications in further understanding the interplay between varying cell types of the BBB in both healthy and pathological states.

Disclosures: S.G. Canfield: None. **M.J. Stebbins:** None. **B.S. Morales:** None. **S.W. Asai:** None. **G.D. Vatine:** None. **C.N. Svendsen:** None. **S.P. Palecek:** None. **E.V. Shusta:** None.

Poster

582. Modeling Psychiatric Disease from iPS Cells

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Topic: A.03. Stem Cells and Reprogramming

Support: Wellcome Trust ISSF Grant (No. 097819)

King's Health Partners

MRC

Royal Society UK

the Brain and Behavior Foundation (formally National Alliance for Research on Schizophrenia and Depression (NARSAD))

Title: Exploring the role of estrogens and inflammation in neurodevelopmental disorders using human induced pluripotent stem cells

Authors: *C. SHUM[1], C. M. PARIANTE[2], P. A. ZUNSZAIN[2], D. P. SRIVASTAVA[2]; [1]King's Col. London, London, United Kingdom; [2]Inst. of Psychiatry, Psychology and Neurosci., London, United Kingdom

Abstract: Multiple studies have shown that estrogens have a powerful effect on cognitive function. At a cellular level, the actions of estrogens are mediated by multiple neuronal signalling pathways, involving classical and non-classical estrogen receptors (ERs). The "classical" pathway involves estrogens binding to ERα and ERβ, transcription factors that regulate target gene transcription via binding to estrogen response element sequences and recruitment of other regulatory proteins. The "non-classical" pathway involves estrogens binding to membrane-bound ERα and ERβ, as well as the G protein-coupled receptor GPER1. Activation of these receptors results in the activation of multiple effectors, leading to various downstream effects, including remodelling of neural circuitry, modulation of synaptic function and connectivity, as well as affecting inflammatory responses. Growing evidence from animal studies have also shown that estrogens have a positive effect on behaviours associated with neurodevelopmental disorders. Recently, clinical studies have demonstrated that adjunct treatment of 17β-estradiol, the main biological estrogen can improve negative, positive and general psychopathological symptoms in female patients with schizophrenia. Moreover, the selective estrogen receptor modulator, Raloxifene has been reported to improved attention and memory in male and female patients with schizophrenia. However, the mechanisms through which estrogens exert their beneficial effects in schizophrenia remain unclear. It has been posited that estrogens may modulate the inflammatory component and/or morphology and synaptic deficits in schizophrenia. In order to explore the potential mechanisms that underlie estrogen's positive effect in schizophrenia, and other neurodevelopmental disorders, we have used human induced pluripotent stem cells (iPSCs) as a cellular model. Using a combination of pharmacological challenges, including exposure to the cytokine Interleukin 1 beta (IL1β) in iPSCs generated from healthy and patient specific cells, we have explored the ability of estrogens to rescue deficits in neuronal morphology and synaptogenesis. To this end, we have performed immunocytochemistry and image analysis to characterise the effects of IL1β or estrogens on neuronal morphology and the expression of synaptic proteins in iPSC-derived cortical neurons. We then explored the effects of estradiol, estrogen receptor agonists and Raloxifene in modulating IL1β-induced phenotypes. Collectively, we hope these data will help us understand how estrogens may confer their positive effects in neurodevelopmental disorders.

Disclosures: C. Shum: None. **C.M. Pariante:** None. **P.A. Zunszain:** None. **D.P. Srivastava:** None.

Poster

582. Modeling Psychiatric Disease from iPS Cells

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Topic: A.03. Stem Cells and Reprogramming

Support: NSF/CBET-1555720

NYSTEM C026415, C026714

Patrick P. Lee Foundation

Title: *In vitro* generation and modification of human neuronal networks and photonic-genetic analyses

Authors: C. BENSON[1], J. KIMM[1], M. NAFARI[1], Z. ZHU[2], D. HUANGFU[2], T. A. IGNATOWSKI[1], P. CLAUS[3], J. M. JORNET[1], M. K. STACHOWIAK, 14214[1], *E. K. STACHOWIAK[1];
[1]SUNY, Buffalo, Buffalo, NY; [2]Sloan-Kettering Inst., New York City, NY; [3]Hannover Med. Sch., Hannover, Germany

Abstract: Ontogeny requires coordinated regulation of multi-gene programs by a plethora of epigenetic signals to execute the cell transitions between self-renewal, proliferative expansion and differentiation. Central function is played by a novel panontogenic mechanism, Integrative Nuclear Fibroblast Growth Factor Receptor 1 Signaling (INFS) *(J. Cell Physiol* 2016, 231: 1199–1218). To analyze the role of INFS in human neural development, we modeled the early formation of the human brain in 3D cerebral organoid cultures developed from hESC lines (HUES8 iCAS9N) and control and schizophrenic iPSCs. It takes approximately one month for the organoids to develop. Stem cells are cultured with various media to first induce the formation of embryoid bodies, next forms neuroectoderm, then neuroepthelial tissue, and finally differentiated cerebral organoids. Once at this step, organoids may stay in the incubator for months growing up to several mm in size.
These cerebral organoids form a ventricular zone with radial astrocytic stem cells, a subcortical zone and cortical layers containing multitudes of neurons and connecting interneurons. The functions of nuclear FGFR1 are investigated by CRISPR/CAS9 mutagenesis of different FGFR1 gene regions important in the generation of the nFGFR1. These mutations have a profound effect on the neuronal morphology and formation of intercellular connections. Our results support the major role of nuclear FGFR1 in the control of human brain development. Given the proposed role of immune activation on neural development, and its involvement in schizophrenia, we are also analyzing the effects of the immune factor, TNFα, on the formation of these neuronal networks.

To analyze the formation of functional neuronal networks, we transfected 2D cultured cells with Channel Rhodopsin (ChR) and used 488 nm laser light to activate the channel and induce calcium signaling across cellular networks, visualized by a calcium dye, Rhodamine-3, imaged at 560 nm. In addition, to improve on this technology, and for future use in the human brain interface, we designed and fabricated nano-photonic actuators (nano-lasers) and receivers (nano-photodetectors) to activate and monitor neuronal networks. Indium Gallium Arsenide Phosphide (InGaAsP) over Indium Phosphide (InP) is utilized as the active semiconductor material and substrate, respectively. In order to minimize the optical signal propagation loss, neuronal networks are being grown on top of the InGaAsP layer (following sterilization by exposure to UV light). These nanophotonic devices may facilitate our ability to monitor and interact with the cellular networks.

Disclosures: C. Benson: None. **J. Kimm:** None. **M. Nafari:** None. **Z. Zhu:** None. **D. Huangfu:** None. **T.A. Ignatowski:** None. **P. Claus:** None. **J.M. Jornet:** None. **M.K. Stachowiak:** None. **E.K. Stachowiak:** None.

Poster

583. Mechanisms of Synapse Formation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 583.01/C14

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

Support: DSRG Graduate Center

Title: Sex differences in synaptogenesis, behavior, and plasticity during critical periods in wild-type and serotonin 1a receptor knockout murine hippocampal development

Authors: *T. BUDYLIN[1,2], S. GUARIGLIA[1], A. MARSILLO[1], D. KERR[1], L. NEUWIRTH[1], D. MCCLOSKEY[1], K. CHADMAN[3], S. SAMMADAR[1], P. BANERJEE[1];
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Abstract: Mood disorders such as anxiety and depression have strong ties to hippocampal serotonin (5-HT) signaling, and are more prevalent in women than in men. Aberrant serotonin $_{1A}$ receptor (5-HT_{1A} R) signaling, an established model of anxiety and depression, is correlated with decreased neurogenesis in the adult dentate gyrus region of the hippocampus. Little is known about neonatal sex differences in neurogenesis, synaptogenesis, behavior and plasticity in the 5-HT_{1A} R KO model. Immunohistochemical staining of immature neurons for colocalized Doublecortin and BRDU, our earlier studies have suggested that 5-HT_{1A}-R signaling through

protein kinase C epsilon (PKCε) augments neuroproliferation in the hippocampus at postnatal day 6 (P6). Our group has also found increased anxiety behavior in adult *5-HT_{1A}-R* (-/-) (KO) females, thus making sex differences in this model intriguing. Verification of this study, as well as sex differences in neurogenesis, quantification was performed with a modified stereological technique in Imaris, a powerful imaging analysis tool. Synaptogenesis and dendrite morphology are also important in brain development and sex differences remain unexplored. Our published transmission electron microscopy (TEM) studies show that hippocampal 5-HT_{1A}-R stimulation at P15 elicits a major boost in synaptogenesis. TEM in the CA1 region was performed on the CA1 region of P11 hippocampi, which is a critical period for hippocampal synaptogenesis in mice. Currently synapses are being quantified and DiI traced dendrites are being analyzed in Imaris with the Filament Tracer tool. Field recordings of the trisynaptic pathway in adolescent brain slices are being analyzed to determine if KO and wild-type mice have Schaffer collateral plasticity differences in brain development. In addition, early developmental milestones are being tested for behavioral differences that can appear postnatally. Together these studies will reveal how aberrant 5-HT_{1A}-R signaling can influence later-life mood disorders, and their sex differences, and may lead to therapeutic strategies for these debilitating disorders.

Disclosures: **T. Budylin:** None. **S. Guariglia:** None. **A. Marsillo:** None. **D. Kerr:** None. **L. Neuwirth:** None. **D. McCloskey:** None. **K. Chadman:** None. **S. Sammadar:** None. **P. Banerjee:** None.

Poster

583. Mechanisms of Synapse Formation

Location: Halls B-H

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Program#/Poster#: 583.02/C15

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

Support: Whitehall Foundation

Alfred P. Sloan Foundation

Edward Mallinckrodt, Jr. Foundation

Title: The role of cadherin diversity in synapse development

Authors: ***M. TAYLOR**, R. BASU, M. E. WILLIAMS;
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Abstract: Variations in cadherin cell adhesion molecule genes are associated with schizophrenia, autism, obsessive compulsive disorder, and learning disabilities; disorders likely

caused by synaptic defects. Cadherins are necessary for synapse development, but their role in linking cell-adhesion to synapse formation is not well understood. Furthermore, there are 18 classic cadherins in mice and humans, and most are expressed in the brain with overlapping, yet distinct, patterns. Thus, individual neurons express multiple cadherins, but the role of cadherin diversity within individual neurons has not been systematically studied. Here we address this fundamental gap by focusing on two cadherins (N-cadherin and cadherin-9) in hippocampal synapse development. We show that both cadherins localize to the same class of synapses, but are differentially trafficked and play distinct roles in synapse development. This evidence supports a model in which diverse cadherins provide unique functions at individual synapses.

Disclosures: **M. Taylor:** None. **R. Basu:** None. **M.E. Williams:** None.

Poster

583. Mechanisms of Synapse Formation

Location: Halls B-H

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

Support: NIH Grant NS062736

NIH Grant T32GM007377

Burroughs Wellcome Career Award; Zito K

Title: The RhoGEF Ephexin5 plays a dual role in regulating the outgrowth of new dendritic spines.

Authors: ***A. M. HAMILTON**[1], J. T. LAMBERT[2], L. K. PARAJULI[3], S. DADAFARIN[2], M. E. GREENBERG[4], S. S. MARGOLIS[5], K. ZITO[2];
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Abstract: The outgrowth of new dendritic spines is closely linked to the formation of new synapses, and is thought to be a vital mechanism underlying behavioral and synaptic plasticity. We recently identified the proteasome as a key regulator of activity-dependent spinogenesis. Here, we examined the role of the RhoGEF, Ephexin5, in connecting proteasome activation to enhanced spine outgrowth. We found that Ephexin5 acts globally as an inhibitor of spine outgrowth, and that it is degraded in an activity-dependent manner in neuronal dendrites and

mature dendritic spines. Remarkably, we also found that a degradation-resistant pool of Ephexin5-GFP accumulated on the dendrite prior to new spine outgrowth, and that removal of Ephexin5 through knockout or RNAi inhibits activity-dependent spine outgrowth induced by global or highly localized stimulation. Our data suggest that Ephexin5 serves a dual role in spinogenesis, acting both as a brake on overall spine outgrowth and as a necessary component in the site-specific formation of new dendritic spines.

Disclosures: **A.M. Hamilton:** None. **J.T. Lambert:** None. **L.K. Parajuli:** None. **S. Dadafarin:** None. **M.E. Greenberg:** None. **S.S. Margolis:** None. **K. Zito:** None.

Poster

583. Mechanisms of Synapse Formation

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Program#/Poster#: 583.04/C17

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

Support: NARSAD

IRSC

Brain and behaviour research foundation

NSERC

Title: Distinct roles of Slitrk2 and Slitrk5 in dopaminergic circuit development.

Authors: ***C. SALESSE**[1,2], J. CHAREST[1,2], H. DOUCET-BEAUPRÉ[1,2], P. DE KONINCK[1,2], M. LÉVESQUE[1,2];
[1]CRIUSMQ, Quebec, QC, Canada; [2]Univ. Laval, Québec, QC, Canada

Abstract: Mesencephalic dopaminergic (mDA) neurons are critically involved in various key functions of the mammalian brain, including voluntary movement, reward, attention, and learning. Dopaminergic circuitry dysfunctions are linked to the development of neuropsychiatric disorders, including obsessive-compulsive disorder (OCD) and OCD-like disorders, such as Tourette's syndrome (TS) and trichotillomania (TTM). The LIM-homeodomain transcriptional factors Lmx1a and Lmx1b are early determinants of the dopaminergic fate and are essential for each step of mDA progenitor differentiation. Using mDA primary cell cultures we found that the loss of function of Lmx1a/b alters dendritic morphology. Spontaneous miniature excitatory and inhibitory post synaptic currents (mEPSC and mIPSC respectively) were also altered in acute slices of Lmx1a/b double conditional mutant (cKO) mice. Using gene expression profiling

experiments in Lmx1a/b cKO mice, we found that Lmx1a/b controls the expression of Slitrk2 and Slitrk5, two members of the Slit and Trk-like (Slitrk) protein family. Gain and loss of function of Slitrk2/5 in mDA cell cultures induced abnormal dendritic morphology and altered synaptic inputs. More specifically, gain and loss of function of Slitrk2 caused a change in the density of excitatory synaptic puncta (PSD95 and VGLUT), but not in inhibitory ones (gephyrin and VGAT). Accordingly, we observed a difference in the frequency, but not in the amplitude, of mEPSCs, after Slitrk2 knockdown or Slitrk2 overexpression. These data suggest a role for Slitrk2 in the formation of functional excitatory synapses. Inversely, gain and loss of function of Slitrk5 induced a modification in the density of inhibitory synaptic puncta but not in excitatory ones. Accordingly, we observed a difference in the frequency, but not in the amplitude, of mIPSCs, after Slitrk5 knockdown or overexpression. These data suggest a role for Slitrk5 in the formation of functional inhibitory synapses. We also investigated the consequences of Slitrk2 and Slitrk5 inactivation on mDA neurons development in vivo and mouse behaviour. Preliminary observations in mice, in which Slitrk2 and Slitrk5 were conditionally knocked-out in mDA neurons using CRISPR-Cas9, confirm our in vitro findings. In addition, analysis of mice lacking Slitk2 in mDA neurons shows that they exhibit OCD-like behaviour. Altogether, our results suggest that Lmx1a/b and Slitrk2/5 are key players in mDA neurons development and synapses formation. This study should contribute significantly to a better understanding of mechanisms involved in OCD-like disorders.

Disclosures: **C. Salesse:** None. **J. Charest:** None. **H. Doucet-Beaupré:** None. **P. De Koninck:** None. **M. Lévesque:** None.

Poster

583. Mechanisms of Synapse Formation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 583.05/C18

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

Title: Ectodomain shedding of nogo-66 receptor by mt3-mmps promotes excitatory synapse formation

Authors: *R. L. SANZ[1], G. FERRARO[1], J. KACEROVSKY[2], E. GOWING[1], J.-F. CLOUTIER[1], K. MURAI[2], A. E. FOURNIER[1];
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Abstract: The functionality of the mammalian central nervous system depends on the formation of an extensively precise network of synaptic contacts assembled during development. The

Nogo-66 receptor (NgR1), a principal mediator of myelin and CSPG-dependent outgrowth inhibition, negatively regulates synapse formation and is important for the closure of the critical period. In the present study, we evaluated the role of synaptic NgR1 proteolysis in the development of excitatory synapses. We identify membrane-type MMPs (MT-MMPs), a membrane-bound MMP subfamily, to be present in the mature rodent brain and to correlate with NgR1 spatiotemporal expression. NgR1 shedding gradually increases in the postnatal cortex and correlates with periods of excitatory synapse development. We characterize MT3-MMP as the protease most likely responsible for synaptic NgR1 processing. Expression of a constitutively shed NgR1 protein increases excitatory synapse formation, while blocking NgR1 cleavage decreases the number of excitatory synapses. Furthermore, treatment with a soluble Ecto-NgR1 (1-358) fragment accelerates excitatory synaptogenesis. Our results identify synaptic Nogo-66 receptor to be subject to MT3-MMP cleavage and raise the interesting possibility that regulated NgR1 cleavage may play a role in the development of excitatory synapses in cortical neurons.

Disclosures: **R.L. Sanz:** None. **G. Ferraro:** None. **J. Kacerovsky:** None. **E. Gowing:** None. **J. Cloutier:** None. **K. Murai:** None. **A.E. Fournier:** None.

Poster

583. Mechanisms of Synapse Formation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 583.06/C19

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

Support: NIH Grant 5R01NS031651

NIH Grant 1R21NS088641

Title: Deciphering the molecular mechanisms that regulate synaptic pruning at the *Drosophila* NMJ

Authors: F. VONHOFF, ***H. S. KESHISHIAN;**
MCDB Dept., Yale Univ., New Haven, CT

Abstract: Neural circuit development generally involves the pruning of off-target synaptic contacts. In mammals low frequency calcium and cyclic nucleotide oscillations refine visual maps in an activity dependent manner. Activity-dependent synaptic refinement also occurs at the Drosophila neuromuscular junction (NMJ). Oscillatory neural activity and presynaptic calcium signaling regulate the motoneuron's response to the muscle-derived chemorepellant Sema2a, acting through the PlexinB receptor. We find that mutations in the Ca-dependent adenylyl

cyclase Rutabaga and the cAMP-dependent phosphodiesterase Dunce also disrupt normal refinement, leading to ectopic neuromuscular synapses on up to 30-40% of the examined muscle fibers. We also demonstrated a role for several molecular players acting downstream in the neuron. These include CaMKII, PKA, and Calcineurin. Loss of function of these genes lead to miswiring with ectopic frequencies of 25-35%. We also have found a role for the protein phosphatase 1 family. PP1 functions as a molecular link between several components of the pathway: PP1 is regulated by Calcineurin and PKA, and dephosphorylates CaMKII. As Ca oscillations are essential for normal refinement in this system, we tested whether cAMP must also oscillate. Optogenetic photoactivation of the bPAC adenylyl cyclase with a 15 s light:150s dark cycle suppressed the miswiring phenotype observed in rut1 adenylyl cyclase mutants. No rescue was observed using activation patterns of shorter or longer cycles (8s light: 80s dark or 30s light: 300s dark). Given that an experimentally induced cAMP oscillation is sufficient to rescue miswiring, we also tested whether cAMP oscillations are necessary. cAMP levels were adjusted to different levels by continuously activating bPAC at various light intensities. A rescue was seen only at a specific intermediate level of activation. bPAC exhibits a constitutive baseline level of activity in the dark. Thus, we examined various levels of transgene expression, using an inducible geneswitch driver. A rescue of the rut1 miswiring phenotype was once again observed with specific levels of bPAC induction. Thus we propose that presynaptic cAMP activity must exist within an optimal range for Semaphorin-dependent synaptic refinement to occur. Finally, we have performed live imaging of growth cone Ca in intact, paralyzed embryos. Ca oscillations are evident in native and ectopic contacts during synaptic development. We are currently testing the effects of Ca dynamics and various mutant backgrounds on filopodial behavior during the innervation of correct and incorrect target muscles.

Disclosures: F. Vonhoff: None. **H.S. Keshishian:** None.

Poster

583. Mechanisms of Synapse Formation

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

Support: : NIH Grant R01 MH067842 (PL)

Simms/Mann Chair in Developmental Neurogenetics (PL)

Title: Alterations in the synaptic proteome of the developing mouse neocortex in the absence of MET signaling

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Abstract: Alterations in the expression of the MET receptor tyrosine kinase have been associated with autism spectrum disorder and Rett syndrome. In the neocortex, MET is expressed in subpopulations of excitatory projection neurons, with peak expression corresponding to the period of process outgrowth and synaptogenesis. In mice, variations in MET signaling modulate dendritic growth, synapse formation, and circuit development and function. Recently, we showed that the MET protein interactome is enriched in proteins implicated in neurodevelopmental disorders. Here, we hypothesize that the cellular and physiological changes due to reduced MET expression will be reflected by changes in the synaptic proteome. To address this, we used quantitative proteomic methods to measure alterations in proteins expressed at the neocortical synapse at the peak of synaptogenesis in the absence of MET signaling. Four independent synaptosome preparations were generated from the neocortex of postnatal day 14 $Met^{fx/fx}/nestin^{cre}$ null mice, in which *Met* is deleted from all neural cells, and from wild type littermates. Two 4-plex iTRAQ (isobaric tag for relative and absolute quantitation) runs were performed by the Vanderbilt University Mass Spectrometry Research Center Proteomics Laboratory, each comparing 2 wild type/null pairs. After filtering to a conservative false discovery rate of <1%, and including proteins with at least two unique peptide measurements, 2728 (run 1) and 3037 (run 2) proteins were identified, with 2452 proteins common to both runs. The protein list contains 157 proteins annotated as synaptic proteins and 502 as mitochondrial proteins, the latter reflecting the high concentration of mitochondria at the synapse. Further, 2195 of the 2452 proteins were identified in an iTRAQ study using synaptosomes prepared from P21 neocortex[1]. The quantitative ratio for each protein was calculated for each null/wild type pair, with a value of 1 indicating that there was no difference between the genotypes. Proteins with an average fold-change <0.8 across the 4 replicates were considered downregulated, while those >1.2 were considered upregulated in *Met* null synaptosomes. A total of 52 proteins were differentially expressed, with 40 upregulated and 12 downregulated. These included proteins involved in proteasome assembly and ubiquitination (6), plasticity (4), cytoskeleton and process outgrowth (8) and signal transduction (5). MET receptor disruption thus results in adaptive changes to the developing synaptic proteome that are consistent with altered function. [1]Moczulska et al., J. Proteome Res. 2014, 13: 4310-4324.

Disclosures: K.L. Eagleson: None. **P. Levitt:** None.

Poster

583. Mechanisms of Synapse Formation

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

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March of Dimes Grant #1-FY11-456

Nellie Ball Trust Research Fund

Title: The γ-protocadherins interact with neuroligin-1 and inhibit its synaptogenic activity

Authors: M. J. MOLUMBY[1], R. M. ANDERSON[2], D. J. NEWBOLD[1], N. K. KOBLESKY[1], A. M. GARRETT[1], D. SCHREINER[1], J. J. RADLEY[2], *J. A. WEINER[1];
[1]Dept. of Biol., [2]Dept. of Psychology, The Univ. of Iowa, Iowa City, IA

Abstract: The α-, β-, and γ-Protocadherins (γ-Pcdhs) are cadherin superfamily adhesion molecules encoded by clustered gene families. The 22 γ-Pcdhs are combinatorially expressed in the brain, and play critical roles in synaptogenesis, dendrite arborization and patterning, and the survival of subsets of neurons. The γ-Pcdhs can interact promiscuously with each other, and with other clustered Pcdhs, in *cis*, but interact strictly homophilically in *trans*. Mice lacking the γ-Pcdhs in the cerebral cortex exhibit severely reduced dendrite arborization (Garrett, et al., *Neuron*, 2012). Recently we demonstrated that γ-Pcdhs promote dendrite arborization *in vivo* through local neuron-neuron and neuron-astrocyte homophilic interactions (Molumby et al., *Cell Reports*, 2016). Though γ-Pcdhs regulate the progression of spinal cord synaptogenesis (Garrett and Weiner, *J. Neurosci.,* 2009), a role for these molecules in cortical dendritic spines and synapses has yet not been examined.

Here, we provide evidence that the γ-Pcdhs negatively regulate synapse formation and spine morphogenesis in forebrain neurons. Mice lacking all γ-Pcdhs in the cortex exhibit significantly increased spine density *in vivo*, while spine density is significantly decreased in mice overexpressing one of the 22 γ-Pcdh isoforms. We thus asked whether the γ-Pcdhs might be inhibitory in an artificial synapse co-culture assay. Indeed, we found that multiple γ-Pcdhs can, when co-expressed in COS cells, strongly inhibit the ability of the synaptic cell adhesion molecule neuroligin-1 to promote presynaptic differentiation in contacting axons. The γ-Pcdhs physically interact in *cis* with neuroligin-1 on dendrites of cultured neurons, and can co-immunoprecipitate neuroligin-1 both *in vitro* and *in vivo*. We present *in vitro* evidence that the interaction between γ-Pcdhs and neuroligin-1 disrupts the latter's binding to its presynaptic partner neurexin1β. Additionally, we show that γ-Pcdh overexpression in cultured hippocampal neurons suppresses the increase in spine density observed upon neuroligin-1 overexpression.

This work suggests a potential new mechanism by which γ-Pcdhs regulate the "choice" between dendrite arbor growth and formation and/or stabilization of dendritic spines and synapses in the developing brain.

Disclosures: M.J. Molumby: None. **R.M. Anderson:** None. **D.J. Newbold:** None. **N.K. Koblesky:** None. **A.M. Garrett:** None. **D. Schreiner:** None. **J.J. Radley:** None. **J.A. Weiner:** None.

Poster

583. Mechanisms of Synapse Formation

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

Support: NIH Intramural Research Program

Title: Inhibitory synapse development: Live imaging of dynamic gephyrin clusters at the convergence of multiple interaction pathways

Authors: *S. BROMLEY-COOLIDGE, W. LU;
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Abstract: While many aspects of excitatory synapse development have been described, inhibitory synapse development has been comparatively understudied. Excitatory synapses do not exist in a vacuum; inhibitory synapses control neuronal excitability and synchronize neural networks, and consequently must be in a dynamic balance with excitatory synapses for proper neural circuit function. Malfunctions of inhibitory synapse development have been shown to lead to many neurological and neuropsychiatric disorders. Therefore, it is imperative to study the mechanisms governing inhibitory synapse development in order to have an informed understanding of neural circuit development and function. Gephyrin is a postsynaptic scaffolding protein analogous to PSD-95 but specific to inhibitory synapses. It is a crucial element of inhibitory synapse development and plasticity; however, the underlying mechanisms converging on gephyrin during synapse development remain largely elusive. Here we used *in vitro* live imaging to examine gephyrin dynamics in developing GABAergic synapses, revealing the various pathways through which inhibitory synapse development is modulated. Dissociated hippocampal and striatal cultures were made from embryonic day 18.5 mice, and imaged at 6-8 days *in vitro*. Various pharmacological manipulations were performed, including inhibition or activation of receptors and channels, as well as the modulation of kinases, cell adhesion molecules, and glial-secreted molecules. In order to systematically evaluate the effect of each

factor on early development, we observed the changes in cell morphology as well as the production, clustering, and motility of GFP- or mCherry-tagged gephyrin clusters at GABAergic synapses. All together, the live imaging of synaptic gephyrin reveals complex dynamics and continuous remodeling important for understanding the mechanisms underlying functional and dysfunctional inhibitory synapse development.

Disclosures: S. Bromley-Coolidge: None. **W. Lu:** None.

Poster

583. Mechanisms of Synapse Formation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 583.10/C23

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

Title: The effect of fluoxetine on c-Fos and synaptophysin expressions in the hypothalamic paraventricular organ during development

Authors: *M. J. CALIBUSO-SALAZAR[1], D. GUNDISCH[1], G. R. TEN EYCK[2]; [1]Pharmaceut. Sci., DKI Col. of Pharmacy, Univ. of Hawaii, Hilo, HI; [2]Biomed. Sci., Home Stryke M.D. Sch. of Medicine, Western Michigan Univ., Kalamazoo, MI

Abstract: Fluoxetine (FLX) is a serotonin re-uptake inhibitor commonly used to treat major depressive disorder. This antidepressant is also the most commonly prescribed drug for pregnant woman who are suffering from depression. Changes in c-*Fos* (transcription factor indicating neuronal activity) and synaptophysin (syn, glycoprotein of presynaptic vesicles; indicative of synaptic plasticity and remodeling) immunoreactivities have been demonstrated in animal, and *in vitro,* studies after chronic or acute FLX treatment. It has been shown that chronic FLX treatment increased c-*Fos* expression in the lateral septal nucleus, the nucleus of the stria terminalis, the medial amygdala, and dorsal raphe; while a decreased was detected in the locus coeruleus. Other studies have shown that chronic FLX treatment induced syn expressions in the adult rat telencephalon and hippocampal neurogenesis. We have previously demonstrated that FLX treatment during embryogenesis affected craniofacial organization. This project has two objectives: 1) determine if chronic treatment of FLX during embryonic development will alter c-*Fos* expression and 2) determine if modulation of serotonin concentration will have an effect on the expressions of syn in the hypothalamic region of the brain. This project utilized the embryo culture technique (Calibuso-Salazar and Ten Eyck, 2015) using the directly developing Puerto Rican coquí frog, *Eleutherodactylus coqui* to assess the effects of FLX on c-*Fos* and syn expressions. The *E. coqui* embryos were directly exposed chronically to FLX concentrations ranging from 0.1µM to 1mM. Immunocytochemistry (anti-cFos and anti-syn) was performed on

sectioned brains to determine changes in c-*Fos* and syn expressions. We found that chronic FLX treatment during embryonic development altered c-*Fos* and syn expressions during brain development. The number of immunopositive cells decreased as we increase the concentration of FLX. The results suggest that modulation of monoamine levels via chronic treatment of FLX during embryonic development leads to reduced dendritic branching and synaptogenesis, thus in turn, reduced chemical synapses.

Disclosures: M.J. Calibuso-Salazar: None. **D. Gundisch:** None. **G.R. Ten Eyck:** None.

Poster

583. Mechanisms of Synapse Formation

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

Support: National Agency of Scientific and Technological Promotion of Argentina (PICT2011-1816, PICT 2013-1145).

Title: Impact of agonist-independent ghrelin receptor, GHSR1a, activity on calcium-mediated signals in primary hippocampal neuron cultures during synaptogenesis.

Authors: *V. MARTINEZ[1], J. LÓPEZ SOTO[2], J. RAINGO[1];
[1]Electrophysiology Lab., IMBICE, La Plata-Tolosa, Argentina; [2]Dept. of Neurosci., Brown Univ., Brown, RI

Abstract: The ghrelin receptor or *growth hormone secretagogue receptor type 1a* GHSR1a is a G-protein coupled receptor highly expressed in several regions of the central nervous system both pre- and post-synaptically. How GHSR1a controls neuronal activity is still poorly understood but multiple mechanisms seem to be implicated. GHRS1a displays two active modes, one dependent on ghrelin binding, its endogenous ligand, and another one termed constitutive active mode independent from ghrelin binding and relying only on GHSR1a expression levels. Recent data from our laboratory suggest that GHSR1a modulates pre-synaptic neurotransmitter release by controlling pre-synaptic voltage-gated calcium channels (Ca_V2) by two independent mechanisms signaling through two different cellular pathways: an acute, Gq-dependent and reversible one, mediated by ghrelin binding to the GHSR1a, and a chronic one, mediated by a Gi/o-dependent reduction of Ca_V2 membrane density due to GHSR1a constitutive activity. Ca_V2 are fundamental structures for neurotransmission, as they allow calcium influx to the pre-synaptic terminal in response to action potentials and thus trigger synaptic vesicles, SVs, fusion to the plasma membrane containing neurotransmitters. In this context, we hypothesize that this

constitutive modulation could be relevant in synapse formation when the establishment of Ca_V2 sub-localization is critical. Thus, we evaluated if GHSR1a expression modifies synapse activity at early stages in primary hippocampal cultured neurons from wild type *versus* GHSR1a knockout mouse embryos by whole-cell patch clamp experiments. We first recorded Ca_V2 total currents and found larger Ca_V2 currents in GHSR1a knockout mice cultures *versus* wild-type mice cultures. We then evaluated miniature spontaneous post synaptic currents, mSPSCs. We also studied evoked inhibitory and excitatory post synaptic currents, IPSCs, at different days *in vitro*. We found that GHSR1a constitutive activity Ca_V currents reduction correlates with smaller IPSCs. Based on our data, we propose that GHSR1a constitutive active mode differentially modulates the timing of spontaneous and action potential-evoked neurotransmitter release.

Disclosures: V. Martinez: None. **J. López Soto:** None. **J. Raingo:** None.

Poster

583. Mechanisms of Synapse Formation

Location: Halls B-H

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Program#/Poster#: 583.12/C25

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

Title: Altered synaptogenesis in a rat model of subcortical band heterotopia

Authors: *F. MARTINEAU[1,2], F. WATRIN[1,2], L. FOURNIER[1,2], E. BUHLER[1,2], F. SCHALLER[1,2], H. KAWASAKI[3], F. SARGOLINI[2,4], B. POUCET[4,2], A. REPRESA[1,2], J.-B. MANENT[1,2];
[1]Inst. de Neurobiologie de la Méditerranée, INSERM, Marseille Cedex 09, France; [2]Aix-Marseille Univ., Marseille, France; [3]Univ. of Kanazawa, Kanazawa, Japan; [4]Lab. de Neurosciences Cognitives, CNRS_UMR7291, Marseille, France

Abstract: Neuronal migration disorders comprise various cortical malformations resulting in neural network alterations, early-onset epilepsy and intellectual disability. These malformations occur when newly generated neurons fail to migrate properly and remain stuck somewhere along their migration route. Although several genes have been identified as causes for neuronal migration disorders, the pathophysiological mechanisms underlying these malformations are still poorly understood. We sought to gain a better understanding of these processes by studying synapse formation in a rat model of one such malformation called subcortical band heterotopia (SBH). This SBH model can be generated by *in utero* electroporation of a plasmid coding for an interfering RNA targeting doublecortin (Dcx), one of the genes responsible for SBH in humans. Through the same genetic method, glutamatergic and GABAergic synapses were labeled with PSD-95-GFP and GFP-gephyrin respectively, two post-synaptic proteins. In affected neurons,

Dcx knockdown lead to a major decrease of both glutamatergic and GABAergic post-synaptic sites densities. Subsequent tri-dimensional reconstruction of these neurons also revealed a drastic simplification of their basal dendritic arborization. Because both the misplacement of the neurons and the gene itself could be the origin of these defects, we are now trying to identify the relative contribution of each of these parameters to this process. To this end, we are currently analyzing two Dcx deficient animal models that do not display any migration defect: a delayed Dcx knockdown rat and the Dcx knockout mouse. Analysis of these models should help unravel the molecular processes by which synaptogenesis is impaired in our SBH rat model.

Disclosures: **F. Martineau:** None. **F. Watrin:** None. **L. Fournier:** None. **E. Buhler:** None. **F. Schaller:** None. **H. Kawasaki:** None. **F. Sargolini:** None. **B. Poucet:** None. **A. Represa:** None. **J. Manent:** None.

Poster

583. Mechanisms of Synapse Formation

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MOST 103-2321-B-001-018

MOST 104-2321-B-001-050

Title: VCP and ATL1 regulate endoplasmic reticulum and protein synthesis for dendritic spine formation

Authors: ***Y.-T. SHIH**, Y.-P. HSUEH;
Academia Sinica, Taipei, Taiwan

Abstract: Imbalanced protein homeostasis, such as excessive protein synthesis and protein aggregation, is a pathogenic hallmark of a range of neurological disorders. Here, using expression of mutant proteins, a knockdown approach and disease mutation knockin mice, we show that VCP (Valosin-Containing Protein), together with its cofactor P47 and the endoplasmic reticulum (ER) morphology regulator ATL1 (Atlastin-1), regulates tubular ER formation and influences the efficiency of protein synthesis to control dendritic spine formation in neurons. Strengthening the significance of protein synthesis in dendritic spinogenesis, the translation

blocker cyclohexamide and the mTOR inhibitor rapamycin reduce dendritic spine density, while a leucine supplement that increases protein synthesis ameliorates the dendritic spine defects caused by Vcp and Atl1 deficiencies. Because VCP and ATL1 are the causative genes of several neurodegenerative and neurodevelopmental disorders, we suggest that impairment of ER formation and inefficient protein synthesis are significant in the pathogenesis of multiple neurological disorders.

Disclosures: Y. Shih: None. **Y. Hsueh:** None.

Poster

583. Mechanisms of Synapse Formation

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

Support: Spanish National Plan SAF2010-21723

ERC Grant PNDYAAR

Title: Identification of Nek7 as a kinase mediating synapse formation in parvalbumin interneurons

Authors: A. J. HINOJOSA[1], *C. KIECKER[2], B. RICO[1];
[1]Dept. of Developmental Neurobio., King's Col. London, London, United Kingdom; [2]MRC Ctr. For Developmental Neurobio., London, United Kingdom

Abstract: Synapse formation is one of the most determining processes in brain circuit wiring. In the cerebral cortex, the two main neuronal populations, glutamatergic pyramidal cells and GABAergic interneurons, connect to each other through completely different types of chemical synapses. Whereas numerous molecular players have proved to mediate synaptogenesis in pyramidal cells, few are the ones identified in interneurons. This study aims, thereby, at revealing some of the differential molecular mechanisms that underlie GABAergic versus glutamatergic synapse formation in the cortex. To achieve that, we used microarrays to obtain the expression profiles of both neuronal types during the synaptogenetic period. To isolate the neurons we took advantage of reporter mice that expressed GFP in either pyramidal cells or interneurons and used fluorescence activated cell sorting (FACS) to get a pure RNA sample of the cell populations. Bioinformatic analysis of the microarray data produced a list of genes differentially upregulated during GABAergic and glutamatergic synaptogenesis.
Among the genes upregulated exclusively during GABAergic synapse formation, we selected

NIMA (Never in Mitosis Gene A)-Related Kinase 7, Nek7. This gene was increased 12 fold during synapse formation and was one of the most specific genes for the interneuron population. Nek7 is a serine/threonine kinase that have been proved to regulate the establishment of the microtubule-based mitotic spindle and to accelerate the dynamic instability of this cytoskeletal component. Microtubules are key regulators of axonal and dendritic arborization in neurons and its role in synapse formation and plasticity is starting to be discovered. Thus, Nek7 was a good candidate to play a role in the postnatal development of interneurons, including synapse formation. Using simultaneously *in situ* hybridization and immunohistochemistry, we demonstrated that Parvalbumin interneurons were the main population expressing Nek7 transcript. To explore whether Nek7 had a function in the process, we carried out loss-of-function experiments in vivo with viruses containing a CRE dependent vector that expressed an shRNA against Nek7. Then, to target these vectors in Nek7 expressing cells, we performed stereotaxic injections in the somatosensory cortex of an Lhx6Cre line, driving Cre recombinase expression in a population of interneurons that includes all Parvalbumin cells. Confocal analysis revealed that loss of Nek7 caused a reduction in the number of parvalbumin synapses onto pyramidal cells. Our findings, unveil a novel protein that is specifically required for inhibitory synapse formation in the cerebral cortex.

Disclosures: **A.J. Hinojosa:** None. **C. Kiecker:** None. **B. Rico:** None.

Poster

583. Mechanisms of Synapse Formation

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Title: Cdk5 activity is required for EphB-mediated synaptogenesis

Authors: ***Y. CHEN**[1,2,3,4], H. WONG[1,2,3], W.-Y. FU[1,2,3], A. FU[1,2,3], N. IP[1,2,3];
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Univ. of Sci. and Technol., Hong Kong, China; [4]The Brain Cognition and Brain Dis. Inst., Shenzhen Inst. of Advanced Technology, Chinese Acad. of Sci., Shenzhen, China

Abstract: Synapses, the specialized structures between neurons, are a vital component of neural circuitry and neurotransmission. The spatiotemporal development of synapses is strictly regulated by well-orchestrated signaling cascades. Aberrant synapse development and function are believed to be associated with neurological disorders such as autism, schizophrenia, and Alzheimer's disease. However, the molecular basis underlying synapse development is not well understood. We previously reported that EphA4 receptor, an Eph receptor tyrosine kinase, induces the retraction of dendritic spines, through a mechanism involving cyclin-dependent kinase 5 (Cdk5). Here, we investigated a novel role of EphB2, another Eph receptor, in the regulation of synapse development and function. EphB2 potentiates the phosphorylation, surface expression, and synaptic recruitment of AMPAR receptor subunit GluA1 in a Cdk5-dependent manner. Interestingly, Cdk5 and its activator p35 form a complex with EphB2 upon ligand-induced activation. Suppressing Cdk5 activity by p35 knockdown or administration of Cdk5 inhibitor perturbed dendritic spine formation and synapse maturation upon EphB2 activation, accompanied by reduced synaptic transmission. Hence, our findings strongly suggest that excitatory synaptogenesis in hippocampal neurons is regulated by EphB2 in a Cdk5-dependent manner.

Disclosures: Y. Chen: None. **H. Wong:** None. **W. Fu:** None. **A. Fu:** None. **N. Ip:** None.

Poster

583. Mechanisms of Synapse Formation

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

Support: Fapesp 2015/04495-0

Title: Nitric oxide and its role in retinal network development

Authors: *L. T. WALTER[1], L. H. E. NISHIO[2], M. I. MÓVIO[3], C. SCHMELTZER[5], G. CERCHIARO[4], A. H. KIHARA[3];
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Abstract: Nitric oxide (NO) is a molecule that accumulates in hydrophobic environments such as membranes and hydrophobic domains of proteins. Moreover, NO has one unpaired electron, which gives a high reactivity characteristic and which allows it to be involved in many physiological and pathological processes. In the central nervous system (CNS), NO has been involved classically involved in synaptic plasticity related processes, such as long-term potentiation. However, the role of NO in the CNS development remains unknown. The aim of this project was to evaluate gene expression (by real-time polymerase chain reaction - PCR) and protein distribution (by immunofluorescence) of the neuronal nitric oxide synthase (nNOS - an enzyme, which are related to the majority production of NO in neurons) in developing and mature retina. We measured NO production levels (by the electron paramagnetic resonance technique - EPR) during retinal development (P0, P5, P10 and P60) and under effect of pharmacological intervention (7-NI blocker). Moreover, we evaluated the effects of NO production on synaptic genes. We observed that mRNA levels of the nNOS are present at all studied ages. Moreover, nNOS seems to be distributed only in specific layers of retina. Specifically, nNOS presence was mainly observed in the inner nuclear layer, where double-labeling experiments and morphological observations revealed two distinct populations of nNOS positive amacrine cells, named as *round* and *balloon* cells. nNOS protein also was detected on inner plexiform layer, which suggest a possible involvement on synaptogenesis. Furthermore, the data obtained from the specific nNOS blocker (7-NI) showed a reduction of synapsin (2^0.41= 0.75-fold-expression; $p<0.05$), synaptophysin (2^0.79= 0.58-fold-expression; $p<0.02$) and connexin45 transcripts (2^0.75= 0.79-fold-expression; $p<0.02$). Taking together, our data suggests that NOS are present in initial stages of postnatal development and, more importantly, that nNOS plays an important role in the formation of electrical and chemical synapses during CNS development.

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Poster

583. Mechanisms of Synapse Formation

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

Support: NIH Developmental Biology Training Grant T32HD007491

Title: The neurodevelopmental disorder gene Kirrel3 regulates target-specific hippocampal mossy fiber synapse development

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Abstract: Synaptic target specificity, whereby neurons make distinct types of synapses only with specific target cells, is critical for normal brain function, yet the mechanisms driving target selection remain poorly understood. We recently showed that Kirrel3 is a synaptic specificity molecule in the mammalian hippocampus. Because alterations in Kirrel3 are associated with intellectual disability and autism, understanding Kirrel3 function can provide insights to cellular and circuit changes underlying these disorders. We demonstrated Kirrel3 is required for proper development of presynaptic mossy fiber filopodia, synaptic structures connecting dentate gyrus (DG) granule neurons to GABAergic neurons in the CA3 region of the rodent hippocampus. Kirrel3 knockout mice have significantly fewer mossy fiber filopodia, suggesting a reduction in DG-GABA synapses. As GABAergic neurons regulate feed forward inhibition onto CA3 neurons, we also observed an increase in CA3 neuron activity in Kirrel3 knockout mice. This suggests that Kirrel3 is required to maintain proper excitation/inhibition balance during brain development. Here we will report on new results using serial blockface scanning electron microscopy (SBEM) to define the precise synaptic defects present in the mossy fiber layer of Kirrel3 knockout mice. We are also determining if patient-associated Kirrel3 point mutations attenuate its function. Together, our results increase our understanding of the links between Kirrel3, synapse development, and neurodevelopmental disorders.

Disclosures: E.A. Martin: None. **D. Woodruff:** None. **R. Rawson:** None. **A. Schneggenburger:** None. **M. Williams:** None.

Poster

583. Mechanisms of Synapse Formation

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FWO Odysseus

FWO postdoctoral fellowship 12N0316N

Title: The sorting receptor SorCS1 controls axonal targeting of neurexin-1alpha via transcytosis

Authors: *J. DE WIT, K. D. WIERDA, K. M. VENNEKENS, L. F. RIBEIRO;
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Abstract: The highly polarized morphology of neurons necessitates mechanisms for sorting proteins to their appropriate cellular compartments. Precise delivery of synaptic surface receptors is essential for the proper establishment of synaptic contacts and the maintenance of neurotransmission. Neurexin-1 (Nrxn-1) is a presynaptic cell adhesion molecule that engages in trans-synaptic interactions with several postsynaptic ligands and plays a key role in the formation, maturation and function of synapses. We recently showed that SorCS1, an endosomal sorting receptor present in the somatodendritic compartment, binds Nrxn-1 and controls its synaptic abundance. However, the mechanism by which somatodendritic SorCS1 regulates the trafficking and synaptic abundance of presynaptic Nrxn-1 remains unclear.
In cultured mouse cortical neurons, Nrxn-1 is polarized to the axonal surface, but is also present in the somatodendritic compartment, consistent with previous reports. We find that blocking endocytosis increases dendritic surface expression of Nrxn-1. Importantly, this manipulation concomitantly disrupts axonal surface targeting of Nrxn-1. Interference with the formation of early and recycling endosomes, but not of late endosomes, results in the same phenotype, indicating that axonal surface expression of Nrxn-1 requires endocytosis and transport via early/recycling endosomes in dendrites. These are hallmarks of transcytosis: axonal proteins are first sorted to the somatodendritic domain and redirected to the axon after endocytosis. Indeed, following experimental release from the ER, Nrxn-1 first traffics to the dendritic surface before appearing on the axonal surface. Furthermore, overexpression of wildtype SorCS1, but not of an endocytosis-defective mutant, removes Nrxn-1 from the dendritic surface and promotes its axonal surface targeting. Together, these data support the idea that SorCS1 governs transcytosis of Nrxn-1. *SORCS1* and *NRXN1* genes have been associated with synaptic disorders, including autism, suggesting that impaired synaptic receptor trafficking contributes to defects in synaptic protein composition and function observed in synaptopathies. Current efforts are directed at determining whether SorCS1-mediated transcytosis of Nrxn-1 sustains presynaptic function.

Disclosures: J. De Wit: None. **K.D. Wierda:** None. **K.M. Vennekens:** None. **L.F. Ribeiro:** None.

Poster

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Title: Gabapentin inhibition of alpha2delta1-mediated synaptogenesis *In vitro* and during adult neurogenesis.

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Abstract: The mammalian brain is remarkably plastic, in part due to the integration of adult-born granule cells into an established hippocampal neural network. Identification of the molecules that control synaptic integration of new adult-born neurons is fundamental to our understanding of neural plasticity in health and disease in the adult brain. Recently, the α2δ proteins have been identified as the receptors for thrombospondins, glia-secreted synaptogenic molecules. Excitatory synapse formation is positively regulated by expression of α2δ during development and *in vitro*, and mutations in α2δ can result in striking neurophysiological phenotypes, such as epilepsy (Barclay *et al.,* Journal of Neuroscience, 2001). Although α2δ proteins have been implicated in neural development (Eroglu *et al.,* Cell, 2009), their contribution to the integration of adult-born neurons is not well understood. Interestingly, a commonly prescribed anti-epileptic and analgesic, gabapentin, binds α2δ with high affinity antagonizing thrombospondin-mediated synaptogenic signaling. Here, using whole cell electrophysiology in organotypic slice cultures, we demonstrate reduction in miniature excitatory post-synaptic currents in gabapentin treated cultures, consistent with a role for α2δ proteins in synaptogenesis. Furthermore, gabapentin significantly reduced dendritic spine density and size in adult-born granule cells *in vivo*, indicating that α2δ contributes to synapse formation during adult neurogenesis. Using *in vitro* and *in vivo* models of neuronal development paired with single-cell morphology and electrophysiology, we hope to decipher the contribution of α2δ proteins to synaptogenesis in adult-born neurons.

Disclosures: K.A. Beeson: None. **G. Westbrook:** None. **E. Schnell:** None.

Poster

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

Support: BRACE grant BIOC SJ1263

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Title: Retromer regulates the retrieval and recycling of neuroligins

Authors: *C. S. BINDA, J. M. HENLEY, K. A. WILKINSON;
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Abstract: Surface proteins are internalised into early endosomes and either sorted for degradation by the lysosome or recycled back to the plasma membrane in a process that for many cargoes is mediated by the retromer complex. For some proteins retrieval and recycling is dependent on an interaction between the PDZ ligand in the cargo protein and the PDZ-domain containing retromer component sortin nexin 27 (SNX27). SNX27 then binds the essential retromer component VPS26 preventing the SNX27 cargo entering the lysosome and therefore rescuing it from degradation.

Retromer dysfunction plays an important role in a growing number of cognitive disorders and the core retromer subunits VPS35 and VPS26 have been reported to be downregulated in the entorhinal cortex in Alzheimer's Disease (AD) patients. SNX27 protein and mRNA is reduced in the cortex of Down's Syndrome (DS) patients. Furthermore, both $VPS26^{+/-}$ knockout mice and $SNX27^{+/-}$ knockout mice have impaired hippocampal-dependent memory and synaptic dysfunction.

Neuroligins are a family of transmembrane cell adhesion molecules that are crucial for synapse function; they bind the presynaptic cell adhesion molecule neurexin to form a trans-synaptic complex which mediates essential pre- and postsynaptic signal transduction.

Here, we show that the PDZ-ligand containing neuroligins 1-3 are cargos for retromer and that they bind SNX27 via a PDZ interaction. Knockdown of SNX27 or VPS35 in cultured cortical neurons significantly decreases total levels of neuroligins 1-3, suggesting that SNX27-retromer is involved in rescuing neuroligins from lysosomal degradation.

These results suggest that the SNX27-retromer dysfunction seen in neuropathological disorders such as AD and DS could lead to impaired synaptic function through reduced recycling of neuroligins back to the synaptic plasma membrane.

Disclosures: **C.S. Binda:** None. **J.M. Henley:** None. **K.A. Wilkinson:** None.

Poster

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

Support: NIH Genetics Training Program Fellowship T32GM007544

Title: Giant ankyrin-G interaction with GABARAP is critical for the formation of GABAergic synapses in mouse somatosensory cortex

Authors: *A. D. NELSON[1], K. K. WALDER[2], V. BENNETT[3], P. M. JENKINS[4];
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Abstract: Several large genome-wide association studies have identified *ANK3* as having a robust association with bipolar disorder, although the mechanisms by which loss-of-function of ankyrin-G contributes to neuropsychiatric disease remains unknown. The giant, 480kDa ankyrin-G (product of the *ANK3* gene) is a critical adaptor protein that organizes membrane proteins at critical excitatory membrane domains, including the axon initial segments and nodes of Ranvier. Recently, we showed that the 480kDa isoform of ankyrin-G also plays an important role in the formation of somatodendritic GABAergic synapses. GABAergic inhibitory signaling is essential for the proper synchronization and function of neuronal networks that underlie cognition, mood, and behavior, and abnormalities in GABAergic interneuron circuitry have been linked to bipolar disorder. Although the 480kDa splice variant of ankyrin-G is a key regulator of GABAergic synapse formation, the exact mechanisms by which ankyrin-G controls inhibitory circuit development are poorly understood. Using cultured neurons, we have shown that giant ankyrin-G interacts directly with $GABA_A$ receptor-associated protein (GABARAP) to inhibit $GABA_A$ receptor endocytosis and stabilize GABAergic synapses. To test the role of this interaction *in vivo*, we generated a mouse model with a W1989R mutation in *Ank3*, which has been shown to completely abolish both ankyrin-G association with GABARAP as well as $GABA_A$ receptor clustering *in vitro.* Interestingly, preliminary data from coronal brain sections from *Ank3* W1989R mice showed the loss of inhibitory synapses on the soma, but not the axon initial segment, of cortical pyramidal neurons in layer II/III of the somatosensory cortex, suggesting a loss of inhibitory synapses from basket cells, but not chandelier cells. In addition, these mice exhibited an overall decrease in PV expression in interneurons, as well as alterations in perineuronal nets (PNNs), which are thought to stabilize GABAergic synapses. As was seen *in vitro*, axon initial segments and nodes of Ranvier are spared in the W1989R mouse, making this

a useful model to study the specific role of basket cell circuitry in normal neuronal activity and how dysfunction in these circuits might contribute to neuropsychiatric disease.

Disclosures: **A.D. Nelson:** None. **K.K. Walder:** None. **V. Bennett:** None. **P.M. Jenkins:** None.

Poster

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

Support: Mallinckrodt Foundation

Title: Non-canonical heterophilic cadherin interactions contribute to layer specific hippocampal plasticity.

Authors: ***R. BASU**[1], X. DUAN[4], M. TAYLOR[2], S. MURALIDHAR[2], J. HUNTER[2], M. YAMAGATA[4], P. J. WEST[3], M. E. WILLIAMS[2], J. R. SANES[4];
[2]Neurobio. and Anat., [3]Dept. of Pharmacol. and Toxicology, [1]Univ. of Utah, Salt Lake City, UT; [4]Ctr. for Brain Sci. and Dept. of Mol. and Cell. Biol., Harvard Univ., Cambridge, MA

Abstract: Single neurons often receive and integrate different types of excitatory inputs at different dendritic locations. Among the critical factors that allow the postsynaptic neuron to differentiate and process these distinct inputs are the unique morphological and electrophysiological properties of these different synapses. Although functional properties of these distinct synapses are often well studied, the underlying molecular signatures that confer these unique properties remain unclear. In the hippocampus, CA1 neurons receive excitatory synaptic inputs from CA3 neurons in two synaptic layers; on their basal dendrites in stratum oriens (SO) and their apical dendrites in stratum radiatum (SR). Interestingly the synaptic plasticity rules are different in these two layers with SO exhibiting higher levels of long term potentiation (LTP) compared to SR. We discovered that loss of the classic cadherin, cadherin-9, results in reduced density of mushroom spines and a reduced level of LTP in the CA1 SO layer while the SR synapses are unaffected. Interestingly, at these synapses we show cadherin-9 is exclusively a presynaptic CA3 molecule. Given that the spine and LTP defects in cadherin-9 knockout mice likely involve postsynaptic mechanisms, we sought to identify postsynaptic binding partner(s) of cadherin-9. We screened hippocampal cadherins and confirmed cadherin-9 heterophilically binds cadherins-6 and 10, which are both expressed in CA1 but not CA3 neurons. Analysis of cadherin-10 knockout (KO) mice reveal that they have spine and LTP

defects specifically in the CA1 SO layer and similar to cadherin-9 knockout mice. Together, our data suggests that cadherins-6, 9, and 10 form a trans-synaptic signaling complex needed for enhanced LTP in CA1 SO. Though classical cadherins are known to mediate adhesion predominantly via homophilic interactions, our work suggests cadherins also use heterophilic interactions to regulate synaptic properties in vivo and cadherin function contributes to establishing complex synaptic identity codes.

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Poster

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

Support: NINDS, NIH intramural research program

Title: An NMDA receptor-dependent mechanism underlies inhibitory synapse development

Authors: X. GU, *W. LU;
NINDS/NIH, Bethesda, MD

Abstract: In the mammalian brain GABAergic synaptic transmission provides inhibitory balance to glutamatergic excitatory drive and controls neuronal output. The molecular mechanisms underlying the development of GABAergic synapses remain largely unclear. Here we report that NMDA-type ionotropic glutamate receptors (NMDARs) in individual immature neurons are the upstream signaling molecules important for GABAergic synapse development, which requires signaling via Calmodulin binding motif in the C0 domain of the NMDAR GluN1 subunit. Interestingly, in neurons lacking NMDARs, while GABAergic synaptic transmission is strongly reduced, the tonic inhibition mediated by extrasynaptic $GABA_A$ receptors is increased, suggesting a compensatory mechanism for the lack of synaptic inhibition. These results demonstrate a crucial role for NMDARs in specifying the development of inhibitory synapses, and suggest an important mechanism for controlling the establishment of the balance between synaptic excitation and inhibition in the developing brain.

Disclosures: **X. Gu:** None. **W. Lu:** None.

Poster

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

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Codei3744

Title: SRPK regulates aggregation of ELKS2 and modulates synaptogenesis in dissociated hippocampal neurons

Authors: V. I. TORRES[1], *N. C. INESTROSA[1], C. C. GARNER[2], P. L. ZAMORANO[3]; [1]Ctr. For Aging and Regeneration (CARE),P. Catholic Univ. of Chile, Santiago, Chile; [2]Neurochemistry and Mol. Biol., Leibniz Inst. for Neurobio., Magdeburg, Germany; [3]Biologia Celular Facultad de Ciencias Biologicas, Pontificia Univ. Catolica de Chile, Santiago, Chile

Abstract: The mechanisms involved in presynaptic active zone formation are not well understood. It has been reported that SRPK79D regulates the aggregation/assembly of the active zone protein Bruchpilot in *D. melanogaster*, a protein with a N-term domain highly homologous to the rat presynaptic scaffold protein ELKS. Here, we explored the role of the SRPK79D rat homologues SRPK 1, 2, 3 in modulating ELKS2 synaptic assembly and their effect in synaptogenesis in rat hippocampal neuronal cultures. In the present study, we employed hippocampal neuronal cultures and heterologous cells HEK293 as platform to asses the oligomerization of ELK2 and its regulation by SRPKs. SRPKs in the CNS show different expression pattern during development: SRPK1 decreases, SRPK2 does not change and SRPK3 increases during synaptogenesis. In neurons, expression of SRPKs is found mainly in the cell soma with a less abundant punctuated distribution in the neurites showing a scanty colocalization with ELKS2 at the growth cones. ELKS2 when expressed in heterologous cells tend to form oligomers, interestingly, SRPK2 and SRPK3 show capacity to disaggregate mRFP: ELKS2 when expressed in HEK293 cells, an effect modulated by the expression levels of SRPKs. Furthermore, expression of SRPK2 and SRPK3 in neurons decreases number of ELKS2 and synaptophysin puncta per length of neurite, but only partially affects Bassoon. These results suggest that the kinases SRPK2 and SRPK3 might be modulating the recruitment of ELKS2 at the active zone, which could also affect other presynaptic proteins

Disclosures: V.I. Torres: None. **N.C. Inestrosa:** None. **C.C. Garner:** None. **P.L. Zamorano:** None.

Poster

583. Mechanisms of Synapse Formation

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

Title: The role of activity and Nova1/2 splicing factors in the development and connectivity of cortical interneurons

Authors: *B. WAMSLEY, X. JAGLIN, G. FISHELL;
Neurosci., NYU Langone Med. Ctr., New York, NY

Abstract: Cortical circuits are complex networks built of hierarchical connections between excitatory neurons and inhibitory interneurons. Cortical interneuron (cIN) function, to balance, excitation to inhibition relies on the various intrinsic electrophysiological features of different classes of INs but also on their distinct abilities to innervate specific postsynaptic partners and target unique subcellular compartments. Although it is now recognized that individual subtypes of INs play unique roles within distinct cortical circuits, the means by which they integrate into circuitry during development is not well understood.
Recent work from our lab and others has demonstrated that early network activity and subsequent modification of gene expression is critical for proper cIN development. However, little is known about the activity-dependent mechanisms underlying establishment of circuit-specific connectivity of cINs. We identified the Nova family of RNA splice factors enriched within cINs during circuit assembly. These factors are particularly attractive, as they have been shown to control the splicing and translation of distinct synaptic pre-mRNAs during development and in response to neuronal stimuli.
Utilizing mouse genetics we are investigating the role of activity and Nova1/2 in the development of cIN connectivity. We find that manipulating cINs activity *in vivo,* maintains the specificity of their contacts but acts to change the density of axonal boutons and profoundly affect Nova1/2 expression within cINs. Conditional loss of Nova1/2 in all INs results in animal lethality due to seizures. To circumvent animal death, we conditionally remove Nova1/2 cell-autonomously and are analyzing any changes in their connectivity via genetic labeling of synapses of individual cINs. Furthermore, we are identifying the genes targeted by Nova1/2 in cINs during synaptogenesis and in response to activity with RNA-seq.
This study will increase our understanding of how specific cIN populations correctly embed into cortical circuitry and how the regulation of RNA transcripts contributes to this process. Understanding these processes carries significant promise as malfunctions in interneuron development and/or synaptic transmission have been implicated in the pathophysiology of many complex neurological disorders including epilepsy, schizophrenia, and autism.

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Poster

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

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Thomas Jefferson University Programmatic Theme Team Award

Title: Ephrin-B3 controls synapse number in an activity independent molecular competition

Authors: N. T. HENDERSON[1], *M. HRUSKA[1], S. LE MARCHAND[2], M. B. DALVA[1]; [1]Dept. of Neurosci., Thomas Jefferson Univ., Philadelphia, PA; [2]Drexel Univ., Philadelphia, PA

Abstract: In the developing brain, neurons must form specific connections in an environment with many potential synaptic partners while also controlling the number of synapses they receive. This precise number and pattern of contacts only emerges after a period of exuberant synaptogenesis, which generates an excess of synapses that are then subjected to further refinement. However, while synaptic sites are over produced during development, connectivity remains sparse, with only a small number of all possible contacts made. Since only a small fraction of the possible connections between neurons exist, how do neurons decide whether to accept or reject potential synaptic contacts?

We propose that differences in eB3 expression levels between neurons regulate the density of synaptic contacts a neuron generates relative to its neighbors though an activity independent molecular competition. A competition requires a winner and loser of a limiting resource. To ask whether eB3 determines winning and losing neurons in a competition for synapses, we used microisland cultures with one or two neurons. In two-neuron islands, cells with wild-type levels of eB3 emerged as winners, forming more synapses than cells in the same island with reduced eB3 expression. Remarkably, EphB2 ecto-domain rescued synapse density following eB3 knockdown in complex cultures and in microislands, suggesting that eB3 competes for EphB2.

To study eB3-mediated competition in genetic model system, we generated eB3 Mosaic Analysis with Double Markers (MADM) mice. These animals have sparsely labeled wild-type ($eB3^{+/+}$) tdTomato+ neurons and eB3 null ($eB3^{-/-}$) GFP+ neurons within an $eB3^{+/-}$ background. Preliminary data indicate that $eB3^{+/+}$ cortical neurons exhibit higher dendritic spine density than

eB3$^{-/-}$ neurons within the same animal. Finally, we used FACS to purify and co-culture eB3$^{+/+}$ and eB3$^{-/-}$ neurons from MADM mice. In these mixed cultures, eB3$^{+/+}$ neurons received a higher proportion of vGlut1 contacts than nearby eB3$^{-/-}$ neurons. Remarkably, blockade of neuronal activity had no effect on the ephrin-B3 dependent differences in synapse density in MADM cultures, suggesting that eB3 may control synapse number independently of activity. Together, these findings define a role for a trans-synaptic protein, ephrin-B3, in controlling synapse number through a novel molecular competition.

Disclosures: **N.T. Henderson:** None. **M. Hruska:** None. **S. Le Marchand:** None. **M.B. Dalva:** None.

Poster

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Title: Haploinsufficiency of the neuronal surface protein MDGA2 enhances excitatory synapse development, alters cortical circuit dynamics and yields behavioral phenotypes consistent with autism in mice

Authors: *S. A. CONNOR[1,2], I. AMMENDRUP-JOHNSEN[2], A. W. CHAN[1,1], Y. KISHIMOTO[4], C. MURAYAMA[5,6], N. KURIHARA[4], A. TADA[4], Y. GE[2], R. YAN[2], J. LEDUE[2], H. MATSUMOTO[6], H. KIYONARI[8], Y. KIRINO[4], F. MATSUZAKI[9], T. SUZUKI[7], D. OJIMA[5], T. H. MURPHY[2], Y. WANG[3], T. YAMAMOTO[5], A. CRAIG[2];

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Abstract: Early brain development is characterized by periods of rapid synaptogenesis which requires highly coordinated trans-synaptic signaling between presynaptic boutons and postsynaptic specializations. Canonical complexes consisting of presynaptic neurexins and postsynaptic neuroligins serve as powerful mediators of synapse differentiation, maturation and activity-dependent modification. Previous studies found that neurexin interactions with neuroligin-2 were decreased in the presence of MAM domain containing glycosylphosphatidylinositol anchor proteins (MDGAs), and MDGA1 was shown to be a negative regulator of inhibitory synapse development. Using mice heterozygous for MDGA2 (*Mdga2*$^{+/-}$; full knockout was perinatal lethal), we characterized MDGA2 function *in vivo*. We discovered that MDGA2 regulates excitatory synapse formation as both the number and function of excitatory synapses was increased *Mdga2*$^{+/-}$ mouse hippocampal slices. In addition, *in vivo* analysis of cortical circuit dynamics through voltage sensitive dye imaging revealed increased cortical excitability in brain midline regions and enhanced cortical region interconnectivity. Behavioral measures yielded data consistent with an autism phenotype including impaired social interactions, increased stereotypies and reduced cognitive function. These results suggest MDGA2 haploinsufficiency enhances excitatory transmission, elevates brain circuit dynamics and compromises cognitive function.

Disclosures: **S.A. Connor:** None. **I. Ammendrup-Johnsen:** None. **A.W. Chan:** None. **Y. Kishimoto:** None. **C. Murayama:** None. **N. Kurihara:** None. **A. Tada:** None. **Y. Ge:** None. **R. Yan:** None. **J. LeDue:** None. **H. Matsumoto:** None. **H. Kiyonari:** None. **Y. Kirino:** None. **F. Matsuzaki:** None. **T. Suzuki:** None. **D. Ojima:** None. **T.H. Murphy:** None. **Y. Wang:** None. **T. Yamamoto:** None. **A. Craig:** None.

Poster

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

Support: EU-FP7 MC-ITN IN-SENS 607616

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Title: Dynamics of the mouse brain cortical synaptic proteome during postnatal brain development

Authors: *M. A. GONZALEZ-LOZANO[1], P. KLEMMER[1], T. GEBUIS[1], C. HASSAN[2], P. VAN NIEROP[1], R. VAN KESTEREN[1], A. SMIT[1], K. LI[1];
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Abstract: Development of the brain involves the formation and maturation of numerous synapses. This process requires prominent changes of the synaptic proteome and potentially involves thousands of different proteins at every synapse. To date the proteome analysis of synapse development has been studied sparsely. Here, we analyzed the cortical synaptic membrane proteome of juvenile [postnatal day 9 (P9), P15, P21, P27], adolescent (P35) and different adult ages (P70, P140, P280) of C57Bl6/J mice. Using a quantitative proteomics workflow we quantified 1560 proteins of which 696 showed statistically significant differences over time. Proteins of the presynaptic active-zone and postsynaptic element showed increased levels over time; proteins involved in protein synthesis or neurite outgrowth generally decreased in abundance. In several cases, proteins from a single functional molecular entity, e.g., subunits of the NMDA receptor, showed profound differences in their temporal regulation, which may reflect specific synaptic development features of connectivity, strength and plasticity. Furthermore, we evaluated the function of Cxadr that showed high expression levels at P9 and a fast decline in expression during neuronal development, suggesting involvement in synapse formation or maintenance. Knock down of the expression of Cxadr in cultured primary mouse neurons revealed a significant decrease in synapse density.

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Poster

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

Support: CHARGE Foundation

Title: Kismet regulates presynaptic vesicle endocytosis and the localization of cell adhesion molecules at glutamatergic synapses

Authors: *F. L. WYGLE-LIEBL, T. L. DELANEY, C. GRIDLEY;
Southern Illinois Univ. Edwardsville, Edwardsville, IL

Abstract: Glutamatergic synaptic transmission is important for a number of behaviors including learning and memory, movement, and visual perception. This neurotransmission relies on the proper localization of cell adhesion molecules and recycling of presynaptic vesicles. We have previously shown that the chromodomain helicase DNA (CHD) binding protein, Kismet (Kis), positively regulates the synaptic localization of glutamate receptors, neurotransmission, and the apposition between glutamate receptors and presynaptic active zones at the *Drosophila* neuromuscular junction. As a transcriptional regulator, Kis likely broadly affects glutamatergic synaptic development. Indeed, we have recently found endocytosis of presynaptic vesicles is deficient at *kis* mutant synapses. Although Kis does not seem to directly regulate transcription of *glutamate receptor* subunits, there is a significant reduction in presynaptic transcript levels of *endophilin B*, *nervous wreck*, and *AP180* in *kis* mutants. Surprisingly, our knock down and rescue experiments suggest that Kis is required in postsynaptic muscles for proper presynaptic endocytosis. Kis may affect endocytosis of presynaptic vesicles and organization of the synapse by regulating the localization of synaptic cell adhesion molecules as *kis* mutants exhibit altered localization of integrins, fasciclin II, and neuroligins. We are currently investigating whether Kis signals by recruiting the chromatin remodeling protein, absent, small or homeotic discs 1 (Ash1) thereby suppressing polycomb group repression and whether Kis influences signaling via the mitogen activated protein kinase (MAPK) pathway.

Disclosures: F.L. Wygle-Liebl: None. **T.L. Delaney:** None. **C. Gridley:** None.

Poster

583. Mechanisms of Synapse Formation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 583.30/D9

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

Title: The kinesin 3 family member Khc-73 is a regulator of the Bone Morphogenetic Pathway and retrograde endosomal traffic

Authors: *E. LIAO, K. TSURUDOME, E. MAKSOUD, P. HAGHIGHI;
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Abstract: Retrograde transport from synaptic terminals to the neuron cell body is an essential process necessary for neuron survival, identity, circuit formation and plasticity. Retrograde transport requires the function of dynein-dynactin motor complexes and anterograde transport is

regulated by kinesin motors. Here we have identified an unusual role for a kinesin 3 motor protein Kinesin 73 (Khc-73) in routing endocytic vesicles to the retrograde pathway at the Drosophila larval neuromuscular junction (NMJ). At the NMJ, synaptic growth and function is regulated by the Bone Morphogenetic Pathway (BMP). During the establishment of synapses, BMP receptors traffic in the retrograde direction to coordinate growth between pre and postsynaptic partners. Our findings indicate that Khc-73 is required for efficient retrograde BMP signalling in motoneurons. Transheterozygous combination of *Khc-73* and BMP transcriptional co-factor *Medea* lead to a significant reduction in the number of presynaptic release sites. Similarly, increased accumulation of phosphorylated form of BMP transcription factor Mad as a result of activation of BMP signaling in motoneurons is fully suppressed when Khc-73 is removed. We did not find any evidence for involvement of *Khc-73* in regulating the degradation of BMP receptors as is the case for several other interactors. Therefore we set out to examine the role of Khc-73 in the regulation of retrograde routing of vesicles further. Interestingly, we found a strong reduction in the dynamics of late endosomes as marked by fluorescently tagged Rab7. Live imaging experiments showed that Rab7 vesicles were stalled along their path from synaptic boutons to the retrograde route along motor axons. In support of this observation, we found abnormally high number of late endosomal multivesicular bodies at synaptic endings using ultrastructural examination. Our result therefore provide a conceptual advance in our understanding of the interaction between dynein and kinesin motor proteins, by revealing a critical role for Khc-73 in routing endosomes onto the retrograde path.

Disclosures: **E. Liao:** None. **K. Tsurudome:** None. **E. Maksoud:** None. **P. Haghighi:** None.

Poster

584. Activity-Dependent Transcription and Responses

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 584.01/D10

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

Title: Kainate receptors regulate maturation of adult-born granule neurons

Authors: *Y. ZHU[1], A. CONTRACTOR[2];
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Abstract: In the adult hippocampus, continuous populations of newborn granule cells (GCs) are generated and undergo successive activity-dependent neuronal maturation and incorporation into the preexisting hippocampal circuitry. Increasing evidence has demonstrated that adult-born neurons in the dentate gyrus can impact numerous cognitive and affective processes such as learning and memory, mood and stress responses. Therefore the mechanisms that control

maturation of adult-born GCs are of relevance to multiple neuropsychiatric disorders. In this study we addressed whether members of the kainate receptor subfamily of glutamate receptors, which are abundantly expressed on adult-born GCs, contribute to activity-dependent maturation of this important neuronal population. To study the maturation of adult-born GCs we used retroviral birth-date labeling of dividing neural progenitors to track their development over a four-week critical period. We compared the maturation of GCs in kainate receptor knockout (GluK2 KO) and control littermate mice using functional and morphological measures including the development of intrinsic membrane properties, dendritic morphology, and development of both inhibitory and excitatory synaptic inputs at various time points after retroviral labeling. We found that the input resistance, as a prime indicator of neuronal maturity, was significantly lower in neurons from the KO mice at 21dpi (WT: 2.46 ± 0.27 GΩ, n = 23; GluK2 KO: 1.53 ± 0.18 GΩ, n = 23, $p < 0.01$) and 28dpi (WT: 1.22 ± 0.18 GΩ, n = 15; GluK2 KO: 0.74 ± 0.07 GΩ, n = 15, $p < 0.05$), suggesting that, surprisingly, loss of GluK2 results in more rapid maturation of adult-born GCs. We also determined the dendritic length and complexity of adult-born GCs as a measure of maturation. The total dendritic length at 21dpi was increased in adult-born GCs in GluK2 KO mice (WT: 1216.4 ± 84.6 µm, n = 5; GluK2 KO: 1707.4 ± 174.9 µm, n = 6, $p < 0.05$) and number of dendritic branch points was also increased at 21dpi (WT: 9.4 ± 0.8, n = 5; GluK2 KO: 13.3 ± 0.9, n = 6, $p < 0.01$) consistent with a more advanced maturation state of adult-born GCs in the GluK2 KO mice. These measures consistently indicate that the rate of maturation of adult-born GCs is accelerated in GluK2 KO mice, which would suggest that kainate receptor signaling normally constrains their development. These results raise many questions about how loss of an excitatory receptor causes faster maturation of these neurons. We are now addressing several mechanistic questions including whether these effects are cell autonomous and whether kainate receptors potentially interact with GABA signaling which is known to play a critical role in adult-born GC maturation.

Disclosures: Y. Zhu: None. **A. Contractor:** None.

Poster

584. Activity-Dependent Transcription and Responses

Location: Halls B-H

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Program#/Poster#: 584.02/D11

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

Support: JSPS KAKENHI 15H02358

JST-CREST

Takeda Science Foundation

Title: Excitation-morphogenesis coupling during corticogenesis: a critical role for L-type voltage-gated calcium channel-driven spontaneous calcium elevations in neurite extension and radial migration

Authors: *S. KAMIJO[1], K. SUZUKI[1], S.-I. HORIGANE[1,2], H. FUJII[1], S. TAKEMOTO-KIMURA[1,2], H. BITO[1];
[1]Univ. of Tokyo, Bunkyo-Ku, Tokyo, Japan; [2]Dept. of Neurosci. I, Res. Inst. of Envrn. Medicine, Nagoya Univ., Chikusa-ku, Nagoya, Japan

Abstract: Calcium signaling is important for various neurodevelopmental processes such as sculpting of neurites, functional wiring and fine tuning of growing networks. Although the existence of spontaneous calcium elevations has been reported in developing neurons, their spatiotemporal pattern, the critical Ca^{2+} sources, and their physiological function have remained elusive. We previously reported that CaMKIα and CaMKIγ decoded local calcium changes and differentially contributed to the elongation of axons and dendrites (Ageta-Ishihara et al. 2009; Horigane et al. 2016). Understanding the ”calcium codes” that govern these CaMK events is thus crucial for deciphering cellular mechanisms underlying cortical development. To address this question, we here combined a membrane-tethered GCaMP with *in utero* electroporation, and imaged spontaneous calcium transients in the neurites of immature layer 2/3 excitatory cortical neurons *in vitro*. We found that spontaneous calcium activity greatly varied in location, duration and amplitude. Though largely stochastic, kymograph-based quantitative analysis revealed that a sizable fraction of local transients were regenerative, and these were termed Spontaneous Regenerative Calcium Transients (SRCaT). Intriguingly, SRCaTs were larger in axons compared to dendrites. Membrane hyperpolarization by overexpressing Kir2.1 inhibited SRCaTs, though tetrodotoxin showed no effects, indicating that SRCaTs were not driven by Na^{+} spikes. Pharmacological experiments suggested a dual calcium source requirement for SRCaT: both Ca^{2+} influx through voltage-gated Ca^{2+} channels (VGCC) and thapsigargin-sensitive IP_3-driven internal stores appeared to be involved. Up- or down-regulation of L-type VGCC resulted in significant bidirectional alteration of axonal outgrowth. Exogenous expression of gain-of-function L-type VGCC mutants harboring single nucleotide variations of Timothy syndrome, an autosomal dominant inherited form of syndromic autism, by *in utero* electroporation, resulted in impaired proper radial migration of layer 2/3 excitatory cortical neurons. Taken together, these lines of evidence suggest an essential role for spontaneous opening of L-type VGCC and SRCaTs in activity-dependent neuronal morphogenesis during corticogenesis.

Disclosures: **S. Kamijo:** None. **K. Suzuki:** None. **S. Horigane:** None. **H. Fujii:** None. **S. Takemoto-Kimura:** None. **H. Bito:** None.

Poster

584. Activity-Dependent Transcription and Responses

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 584.03/D12

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

Title: Distinct mechanisms control the start and end of experience-dependent plasticity at the VPm relay

Authors: *L. PAN, J. YANG, Q. YANG, H. LOU, S. DUAN, H. WANG;
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Abstract: Synapse plasticity, which refers the ability of synapses to be strengthened or weakened in the central nervous system, plays a critical role in normal brain function including learning and memory. Recent studies have found in addition to cortical area, thalamus is also plastic during a particular time window. However, the underlying mechanisms are still unclear. Here, by using *in vitro* whole-cell patch recording in acute brain slices we found that the precise critical period at the VPm relay synapse was from P11 to P14. Whisker deprivation later than P11 or earlier than P14 significantly altered synaptic properties within 24 hrs. Further studies found that between P10 and P11, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR)-EPSCs, N-methyl-D-aspartate receptors (NMDAR)-EPSCs were not significantly changed, whereas NMDAR decay constant and sensitivity to ifenprodil, a selective NR2B-containing NMDAR blocker were largely altered. These results indicate that more NR2A-containing NMDA receptors were recruited to VPm relay synapse at P11 when compared with that at P10. We next found that intraperitoneal injection of PEAQX, which selectively blocked the function of NR2A-containing NMDA receptors, delayed the maturation of VPm relay synapse. These results suggest that the recruitment of NR2A containing NMDARs may responsible for the entering of critical period at the VPm relay synapse. During the end of critical period, we did not observe any changes of synaptic properties occurred between P14 and P15. Through the method of calcium imaging, we found that the timing of the switch from depolarizing to hyperpolarizing GABA show great changes between P14 and P15 in VPm neurons. These data demonstrate that the maturation of GABAergic inhibition is concurrent with the ending of critical period.

Disclosures: **L. Pan:** None. **J. Yang:** None. **Q. Yang:** None. **H. Lou:** None. **S. Duan:** None. **H. Wang:** None.

Poster

584. Activity-Dependent Transcription and Responses

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

Support: NIH Grant NS080979

Ellison Medical Foundation

Title: Transcriptional response to single exposures to physical exercise

Authors: *C. CHATZI, Y. ZHANG, R. H. GOODMAN, G. L. WESTBROOK;
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Abstract: Neurons have the remarkable ability to process and respond to complex stimuli such as physical exercise and changes in an organism's external environment. In rodents such experiences increase hippocampal synaptic plasticity and neurogenesis, spatial memory and pattern separation. However there is still little information on the signaling pathways that underlie these experience-dependent changes. The majority of *in vivo* studies have singled out transcriptional regulators without being able to provide a model for understanding the behavior of gene networks engaged upon synaptic activity, while they also were limited by temporal and spatial resolution. We have developed a novel method for *in vivo* analysis of the transcriptional cascades of discrete populations of dentate granule cells activated by physical exercise within a specific time window.

Immediate early response gene *Fos* expression is undetectable in quiescent cells but can be rapidly and transiently induced by external stimuli such as exercise. $cFos^{cre/ERT2/Luo}$ transgenic mice use the connection between *Fos* expression and neuronal synaptic activity in order to permanently label neurons that are active over a short time period. We used a virus expressing a CAG promoter followed by a loxP-flanked ('floxed') stop cassette-controlled Histone2B Monomeric Red Fluorescent Protein (H2B-mRFP) that was injected stereotaxically into the dentate gyrus of $cFos^{cre/ERT2/Luo}$ mice. Two weeks post viral injections, mice were injected with tamoxifen while caged with a running wheel for 2 hrs or were undisturbed in their homecage, and then mice were sacrificed at several timepoints following tamoxifen administration. Voluntary exercise resulted in a significant increase of the activated mRFP +ve cells in the DG in comparison to mice left in the homecage. mRFP labeled (i.e. activated) and non-activated granule cells from exercised mice were subsequently excised from non-fixed intact tissue cryosections using laser capture microdissection and pooled in batches of 100-150 cells. RNA from microdissected cells was reverse transcribed and processed for RNASeq library construction. Additionally, we investigated how exercise-induced neuronal activity regulates synaptogenic processes including synapse development and maturation. Our experiments provide

cell and temporal specific approach to the influence of exercise-induced synaptic activity on neuronal gene expression.

Disclosures: C. Chatzi: None. **Y. Zhang:** None. **R.H. Goodman:** None. **G.L. Westbrook:** None.

Poster

584. Activity-Dependent Transcription and Responses

Location: Halls B-H

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Program#/Poster#: 584.05/D14

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

Support: Grants-in-Aid for Sasakawa Scientific Research No. 28-411

Title: Neuronal inactivity dependent-undifferentiation in primary visual cortex

Authors: *H. SHIN, H. D. KAWAI;
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Abstract: Recent studies reported that sensory deprivation regulated cell proliferation of NG2-expressing glia cells in somatosensory cortex (Mangin et al., 2012, Nat. Neurosci. 15, 1192-1194) and that of neural progenitor cells in the tectum (Sharma and Cline, 2010, Neuron, 68, 442-455). It is unclear whether proliferating cells existed in primary visual cortex (V1), and whether and how visual deprivation (VD) regulates proliferation or differentiation. Here we investigated these issues in V1 of binocularly enucleated juvenile mice.
We enucleated at postnatal day 15 (P15), the time of eye opening, and examined if there are proliferating cells after 7- or 10-day VD. To examine for the presence of proliferating cells, BrdU was administrated daily for 3 days from P19 to P22 (P19-22) or from P22 to P25 (P22-25). The cell density of BrdU positive (BrdU+) cells was similar at P22 and P25 in control mice. Enucleated mice however showed a general increase in the density of BrdU+ and Ki67 positive (Ki67+) cells (i.e., undifferentiated cells) throughout the cortex with about 3-fold increase in layer 6 at P25. Enucleation also increased the proportion of Ki67+ cells among BrdU+ cells throughout the cortex, especially in layers 2/3, at P25, although the total BrdU+ cell density did not change. The cell density and the proportion of undifferentiated doublet cells with their distance of 10 μm or less also increased at P25, suggesting an increase of recently divided cells due to the lack of neuronal activities during P22-25, which corresponds to the beginning of the critical period for ocular dominance plasticity. Only a very few newly proliferated cells were apoptotic as determined by the presence of Caspase-3. These data suggest that the lack of neuronal activities increases undifferentiation of proliferating cells during the important period

of synapse formation.
Next, we examined the cell type of BrdU+ cells with several markers at P25. The BrdU+ cells lacked the co-immunostaining of GAD67 (inhibitory neuronal marker), β-tubulin (immature neuron marker), NG2 (NG2 positive glia cell or oligodendrocyte progenitor cell marker), or GFAP (mature or reactive astrocyte marker). In the meantime, ~52% of BrdU+ cells co-expressed S100β in control mice, which increased to ~75% following enucleation. This increase was due to the increase in BrdU+Ki67+ cells from ~17% to ~31% without changing BrdU+Ki67- cells, suggesting that enucleation increases astrocyte progenitor cells without affecting differentiating astrocytes.
Overall, these data suggest that VD following enucleation at the time of eye opening promotes the undifferentiated state in astrocyte progenitor cells in developing V1.

Disclosures: H. Shin: None. **H.D. Kawai:** None.

Poster

584. Activity-Dependent Transcription and Responses

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 584.06/D15

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

Support: NS40296

Title: Examining synaptic competition between tonic and phasic motor neurons at *Drosophila* neuromuscular junctions

Authors: *N. A. APONTE-SANTIAGO[1], J. LITTLETON[2];
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Abstract: Structural plasticity induced by neuronal competition is a common feature of developing nervous systems, and alterations in this process may contribute to neurodevelopmental disorders. Although several mammalian systems have been used to study activity-dependent synaptic competition, the molecular mechanisms underlying this process are still being elucidated. The *Drosophila* larval neuromuscular junction (NMJ), a model glutamatergic synapse with excellent genetic accessibility, may provide a useful system to study synaptic competition and compensation. Most larval muscle fibers are innervated by at least two motor neurons, a type Ib "tonic" motor neuron and a type Is "phasic" motor neuron. The Ib neuron innervates a single muscle, while Is neurons innervate a subset of muscles to coordinate contraction of distinct muscle groups. If and how the postsynaptic muscle can differentiate

between these two inputs, and how it might preferentially regulate them, is unknown. We are examining if Ib and Is motor neurons compete for synaptic drive to the postsynaptic cell by creating an input imbalance through genetic manipulation of one of the two neurons. To determine if synaptic competition at the NMJ occurs, we genetically altered the Ib neuron innervating muscle 1 (MN1-Ib). Surprisingly, complete elimination of MN1-Ib did not result in changes in innervation of the corresponding 1s neuron. In contrast, decreasing activity of the MN1-Ib neuron (rather than ablating it) using *vglut* RNAi or by expressing tetanus toxin light chain resulted in changes of the co-innervating 1s neuron. Additional studies are underway to further test the ability of the *Drosophila* NMJ to display synaptic competition. In addition, we are examining if plasticity is manifested in a synapse-specific fashion only at the target muscle where 1b motor neuron activity is altered, or across all muscles innervated by the 1s neuron.

Disclosures: N.A. Aponte-Santiago: None. **J. Littleton:** None.

Poster

584. Activity-Dependent Transcription and Responses

Location: Halls B-H

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

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Title: The role of cyclic adenosine monophosphate and apolipoprotein A1 on myopic eye growth

Authors: *C. H. TO[1], R. K.-M. CHUN[2], C. W. DO[2];
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Abstract: OBJECTIVE. To investigate the effects of cAMP on eye growth through its interaction with apolipoprotein A1 (ApoA1) in young chicks. METHODS. Four groups of chicks were examined. Group 1: Chicks received 8-Br-cAMP (0.1 mM or 1 mM) and were fitted with -10 diopter (D) lenses on both eyes, whereas chicks in group 2 (0.1 mM 8-Br-cAMP) wore plano lenses over both eyes. 8-Bromo-cAMP (8-Br-cAMP) was injected intravitreally to the right eyes of 8-day old chicks for 4 consecutive days and control eyes received vehicle only. The levels of

retinal cAMP and ApoA1 were measured in another two groups of chicks wearing -10 D (group 3) and +10 D lenses (group 4) on their right eyes for 3 days respectively (plano lenses were worn by left eyes). RESULTS. Although, Br-cAMP had little effect on normal eye growth, it significantly inhibited the development of lens-induced myopia (group 1: 0.1 mM versus vehicle: +1.71 ± 1.22 D versus -8.00 ± 2.19 D; 1 mM versus vehicle: +1.38 ± 1.34 D versus -9.96 ± 1.14 D, mean ± SEM, $P < 0.01$ for both); 1 mM, but not 0.1 mM 8Br-cAMP increased retinal ApoA1 expression ($P < 0.01$). Both retinal cAMP and ApoA1 levels were significantly increased only in hyperopic eyes (group 4). CONCLUSIONS. 8-Br-cAMP significantly inhibited myopic growth as induced by optical lenses. Cyclic AMP activates retinal ApoA1 expression in the process and the present data suggested a possible interplay between ApoA1 and cAMP in regulating eye growth.

Disclosures: C.H. To: None. **R.K. Chun:** None. **C.W. Do:** None.

Poster

584. Activity-Dependent Transcription and Responses

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

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Title: The non-classical MHCI Qa-1 is a novel regulator of experience dependent plasticity in visual cortex

Authors: *K. S. CHEW, C. J. SHATZ;
Biol., Stanford Univ., Stanford, CA

Abstract: For the nervous system to translate experience into memory, lasting structural change at synapses must occur. This requirement is evident during critical periods of activity-dependent development during which early experience sculpts connections to establish adult circuits via strengthening of selected subsets of synapses and weakening and eliminating others. While activity-dependent mechanisms are known to be essential, molecular mechanisms underlying how activity refines connections are not well understood.

Research has identified a surprising role for several classical major histocompatibility complex class I (MHCI) proteins in activity-dependent refinement of neural networks. The presence of MHCI protein in neurons is notable because GWAS studies have linked polymorphisms in the MHC genomic cluster with several neuropsychiatric disorders including schizophrenia. There are more than 50 MHCI alleles, yet to date only two have been studied in brain. The MHCI family is subdivided into two groups: classical MHCIs are highly polymorphic, players in adaptive immunity and interact with T-cell receptors. In contrast, non-classical MHCIs are less polymorphic and interact with inhibitory and activating innate immune receptors.
Here we report that the mouse non-classical MHCI, Qa-1, is expressed in neurons and specifically enriched in layer 6 cortical neurons, as well as a subset of cerebellar Purkinje neurons. To explore the functional significance of neuronal Qa-1, we investigated its role in ocular dominance plasticity (OD) in visual cortex. During the critical period, OD plasticity in Qa-1 KO mice is enhanced following a period of monocular visual deprivation. In the mouse immune systems, Qa-1's cognate receptor is a CD94/NKG2 heterodimer, specifically CD94/NKG2A, C, or E. These putative receptors are also expressed in brain, suggesting that Qa-1 may act through a CD94/NKG2 heterodimer to regulate ocular dominance plasticity. Together, these observations point to a broadening role for both classical and non-classical neuronal MHCI proteins in plasticity and neural circuit refinement in cerebral cortex, and imply a related function for Qa-1 in cerebellar Purkinje cells.

Disclosures: K.S. Chew: None. **C.J. Shatz:** None.

Poster

584. Activity-Dependent Transcription and Responses

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 584.09/D18

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

Title: The role of serotonergic neurons in spontaneous activity of the developing mouse hindbrain

Authors: *L. E. HOOD[1], M. BOSMA[2];
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Abstract: Early in neural development, cells in the ventral midline of the mouse hindbrain spontaneously depolarize in the absence of external stimulation. These electrical events are dependent on t-type calcium channels and trigger intracellular calcium influx, which we measure with calcium imaging techniques. The spontaneous activity (SA) begins as isolated events at embryonic day (E) 10.5 and builds into synchronized waves of activity that repeatedly propagate

along the entire midline by E11.5. During this same developmental window, serotonin neurons are specified and differentiate in two columns flanking the spontaneously active midline. Pharmacology studies have implicated the involvement of serotonin in the initiation of spontaneous events; no other transmitter systems are required for the spontaneous activity. In this study, we use serotonin-receptor-specific pharmacological agents to further probe the role of serotonin in the generation of activity. We use quantitative real time PCR to examine the relative levels of expression of a range of serotonin receptors including 5HT2A and 5HT2C. In addition, we have examined the effect of blocking serotonergic signaling on the morphology of the serotonergic cells. Hindbrains cultured in antagonists specific to various serotonin receptors for 24 hours show a significant reduction in the number of 5HT+ cells. However, the same reduction in cell number does not occur when the SA is halted via blocked calcium channels. This suggests serotonin signaling is crucial for appropriate development of serotonergic neurons within the hindbrain.

Disclosures: L.E. Hood: None. **M. Bosma:** None.

Poster

584. Activity-Dependent Transcription and Responses

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

Support: F32NS086270

P50MH106933

Title: Transcriptional profiling of human neuronal activity-regulated gene expression

Authors: *G. BOULTING[1], B. ATAMAN[1], D. HARMIN[1], M. YANG[1], V. BEREZOVSKII[1], E.-L. YAP[1], I. SPIEGEL[1], M. PLETIKOS[2], N. SESTAN[2], C. WALSH[3], M. LIVINGSTONE[1], M. E. GREENBERG[1];
[1]Neurobio., Harvard Med. Sch., Boston, MA; [2]Dept. of Neurosci. and Kavli Inst. for Neurosci., Yale Sch. of Med., New Haven, CT; [3]Boston Children's Hospital, Harvard Med. Sch., Boston, MA

Abstract: Neuronal activity regulates aspects of the structure and function of brain circuitry in part through the induction of calcium-dependent programs of gene expression. While neuronal activity-regulated genes have been profiled and shown to be important for neurological faculties, including memory and learning in model organisms, little is known about such gene expression

programs in human neurons. We have transcriptionally profiled depolarized human fetal brain cultures, and identified a new neuronal activity-dependent secreted factor Osteocrin (OSTN). *OSTN* is enriched in the neocortex of human and macaque, but is not expressed in mouse neurons. Our gene expression studies suggest that *OSTN* has been evolutionarily repurposed through the acquisition of a primate-specific activity-regulated neuronal enhancer, and our functional studies show that OSTN restricts activity-dependent dendritic growth in human neurons. These findings suggest that subtle sequence changes in cis-regulatory elements allow for neuronal activity-regulated gene programs to be tailored for lineage-specific features of brain plasticity and development.

Disclosures: G. Boulting: None. **B. Ataman:** None. **D. Harmin:** None. **M. Yang:** None. **V. Berezovskii:** None. **E. Yap:** None. **I. Spiegel:** None. **M. Pletikos:** None. **N. Sestan:** None. **C. Walsh:** None. **M. Livingstone:** None. **M.E. Greenberg:** None.

Poster

584. Activity-Dependent Transcription and Responses

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

Support: NIH grant P50MH106933

Fellowship from The Ellen R. and Melvin J. Gordon Center for the Cure and Treatment of Paralysis

Title: Evolutionary repurposing of an activity-regulated factor in primate neocortex

Authors: *B. ATAMAN[1], G. L. BOULTING[1], D. A. HARMIN[1], M. G. YANG[1], E.-L. YAP[1], I. SPIEGEL[1], M. PLETIKOS[2], M. CHAHROUR[3], N. SESTAN[2], C. A. WALSH[4], V. K. BEREZOVSKII[1], M. S. LIVINGSTONE[1], M. E. GREENBERG[1];
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Abstract: Experience-driven programs of neuronal gene expression have been shown to regulate the plasticity of brain circuitry in model organisms. However, it is not known if there are neuronal activity-regulated genes that control features of brain development and function that are unique to primates, including humans. We identified a new activity-dependent secreted factor Osteocrin (OSTN) by transcriptional profiling of human fetal brain cultures. While *Ostn* is exclusively expressed in bone and muscle of mouse, *OSTN* is enriched in the neocortex of human

and macaque. We find that *OSTN* gene has been evolutionarily repurposed in the primate lineage through the acquisition of a new neuronal activity-regulated enhancer. Functional studies showed that *OSTN* restricts activity-dependent dendritic growth in human neurons. These findings suggest that modification of neuronal activity-regulated gene programs, via the evolution of cis-regulatory elements, may contribute to lineage-specific features of brain plasticity.

Disclosures: **B. Ataman:** None. **G.L. Boulting:** None. **D.A. Harmin:** None. **M.G. Yang:** None. **E. Yap:** None. **I. Spiegel:** None. **M. Pletikos:** None. **M. Chahrour:** None. **N. Sestan:** None. **C.A. Walsh:** None. **V.K. Berezovskii:** None. **M.S. Livingstone:** None. **M.E. Greenberg:** None.

Poster

584. Activity-Dependent Transcription and Responses

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 584.12/D21

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

Support: Epigenomics Flagship Project EPIGEN MIUR-CNR

Telethon project GGP1114

Title: Regulation of transcriptome during postnatal development in mouse visual cortex

Authors: ***D. NAPOLI**[1,2], R. MAZZIOTTI[3], F. CACCIANTE[2], M. GENNARO[1], E. PUTIGNANO[1], P. TOGNINI[4], N. CEGLIA[4], C. MAGNAN[4], P. BALDI[4], T. PIZZORUSSO[3]; [1]Inst. of Neuroscience, CNR, Pisa, Italy; [2]BIO@SNS lab, Scuola Normale Superiore, Pisa, Italy; [3]Dept. of Neuroscience, Psychology, Drug Res. and Child Hlth. NEUROFARBA Univ. of Florence, Florence, Italy; [4]Ctr. for Epigenetics and Metabolism, Univ. of California, Irvine, CA

Abstract: The mechanisms at the basis of cortical postnatal development are still poorly understood. It has been proposed that cortical development derives from a delicate interplay between innate and experience-dependent factors converging at the level of the regulation of gene expression. In this context, miRNAs could be molecular “chisels” used to sculpt the transcriptome during development. To understand their role in visual cortical development we performed sequencing analysis of miRNAs and mRNAs from the visual cortex of the mouse at P10 and P28, two ages corresponding to the beginning and completion of the functional development of visual cortical neurons. We found that a dramatic age-dependent regulation of the transcriptome affecting a number of KEGG pathways. Intriguingly we found that age-dependent regulation of miRNAs was paralleled by regulation of their putative targets in an

opposite manner, suggesting that miRNAs shape the cortical transcriptome during this period of development. Functional validation of the role of the regulated miRNAs will elucidate the relevance of this regulation for cortical development.

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Poster

585. ADHD and Other Behavior Disorders

Location: Halls B-H

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Program#/Poster#: 585.01/D22

Topic: A.07. Developmental Disorders

Support: NIH/NIMH grant 5R01MH104313

Title: N-acetyl aspartate is related to sensory processing challenges in children: A preliminary study

Authors: ***N. M. KLEINHANS**[1], N. CORRIGAN[1], M. REILLY[1], L. BARRERA[1], M. REITER[1], A. ESTES[2], T. ST. JOHN[2], T. RICHARDS[1], S. DAGER[1];
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Abstract: Children with sensory processing challenges (SP) show atypical reactions to everyday sensory information. Current hypotheses regarding the biological basis of SP implicate cortical regions involved in primary sensory processing and multimodal sensory integration. The role of the amygdala, a subcortical structure which receives inputs from all sensory modalities, is unknown. We investigated whether N-acetylaspartate (NAA), a marker of neuronal integrity, was different in children with SP compared to typically developing (TD) children, and whether NAA levels corresponded to individual differences in parent reported sensory symptoms. Children with SP had a significantly elevated Sensory Profile score on at least 1 of 4 quadrants: Seeking/Seeker, Avoiding/Avoider, Sensitivity/Sensor, Registration/Bystander. Autism spectrum disorder was ruled out in all participants. Data collection is ongoing. The sample included 10 children with SP and 12 age- and IQ-matched TD controls between 8 and 12 years of age. 3D MPRAGE and magnetic resonance spectroscopic (MRS) data were acquired on a 3T Philips Achieva system. Single-voxel MRS data were acquired from a left amygdala-hippocampal region and a left lateral superior cerebellar region using a PRESS sequence (TE=35ms, TR 2000ms, 2048 complex time points, spectral width 2000 Hz, 32 avgs, voxel size 30x30x30mm). NAA concentrations were estimated using LCModel, referenced to water, and corrected for CSF

partial volume effects. Independent t-tests and bivariate correlations between NAA concentrations and scores on the Sensory Profile were run using SPSS. Results: The SP group had significantly higher NAA than the TD group in the amygdala-hippocampal region (p = .007), but not in the cerebellar region (p = .543). Correlations were run on the combined sample (N=22). Higher amygdala NAA was correlated with significantly more sensory symptoms in 5/6 sensory sections (not oral) and all 4 Quadrants. The most robust correlations (p < .005) were observed in the auditory (r = .545), touch (r = .690), body position (r = .593) and movement (r =.773) sections and the Seeking/Seeker (r=.749) and Registration/Bystander (r =.704) Quadrants. No significant correlations were observed in the cerebellum (p vals ranged from .225 to .959). In this preliminary study, higher levels of amygdala-hippocampal NAA were associated with more severe sensory challenges, indicating that atypical sensory processing may be related to increased dendritic arborization and synaptic connectivity. Elevated NAA does not appear to be a generalized effect throughout the brain in SP, as group differences were not observed in our cerebellar control region.

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Poster

585. ADHD and Other Behavior Disorders

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Topic: A.07. Developmental Disorders

Support: MYRG2014-00093-FHS

MYRG 2015-00036-FHS

FDCT 026/2014/A1

FDCT 025/2015/A1

Title: Alternation of resting-state brain connectivity in the orbitofrontal & the dorsolateral prefrontal cortices of heroin addicts & its relationship with anxiety: fNIRS study

Authors: ***H. IEONG**, Z. YUAN;
Univ. of Macau, Macau, Macao

Abstract: Drug addiction is widely linked to the orbitofrontal cortex (OFC) and the dorsolateral prefrontal cortex (dlPFC) which is essential for regulating reward-related behaviors, emotional and inhibitory responses, and anxiety. Over the past 20 decades, neuroimaging has provided significant contributions revealing the functional and structural alternations in the brains of drug addicts. However, the underlying neural mechanism in the cortices and their correlates of drug addiction and anxiety still need further elucidation. In this study, we examine the local network dynamics in the OFC and dlPFC regions through resting-state functional connectivity (rsFC) by using functional near infrared spectroscopy (fNIRS) from eight chronic users in the heroin-dependent group (HD) and seven normal subjects in the control group (CG). We discovered that the HDs manifested enhanced interhemispheric correlation and functional connectivity across the OFC regions. Moreover, the small-worldness was identified in the HD brain networks. We suggest that the individuals under chronic heroin influence carry more complex local brain networks than normal individuals. Alongside the abnormal robustness, our findings also revealed that there was a dissociation in the functional connectivity between the left inferior frontal gyrus and other regions correlated with anxiety. This work may pave a new insight into the neural adaptations resulting from chronic opiate intake.

Disclosures: H. Ieong: None. **Z. Yuan:** None.

Poster

585. ADHD and Other Behavior Disorders

Location: Halls B-H

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Program#/Poster#: 585.03/D24

Topic: A.07. Developmental Disorders

Title: The neural underpinnings of Learning Disabilities (LD) - in search of biomarkers

Authors: N. ALAHMADI[1], *L. JANCKE[2];
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Abstract: Learning disability (LD) is diagnosed when an individual's achievement on individually administered, standardized tests in reading, mathematics, or written expression is substantially below that expected for the individual's age, schooling, and level of intelligence. A major problem in diagnosing LD is the strong cultural and educational influence on the diagnosis. Thus, objective and easily to apply methods would be helpful for LD diagnosis. In this paper we demonstrate first findings of our project to establish neurophysiological biomarkers helping to diagnose LD. Here we have used electroencephalographic (EEG) measurements during resting state condition in LD children and in healthy control children (HC).Using the

group independent component analysis (gICA) model to identify substantial differences between LD and HC children in terms of their spectral EEG profiles we obtained the following findings: (a) there was substantial slowing of EEG oscillations, especially for gICs located in frontal scalp positions; (b) the estimated intracortical sources of these gICs were mostly located in brain areas involved in the control of executive functions, attention, planning, and language; and (c) those LD children suffering predominantly from verbal problems (LD-Verbal) demonstrated substantial differences in EEG oscillations in language-related brain areas. The general pattern of atypical neurophysiological activation found in LD children suggests that they suffer from neurophysiological dysfunction in brain areas involved with the control of attention, executive functions, planning, and language functions. LD-Verbal children also demonstrate atypical activation, especially in language-related brain areas. Using these neurophysiological measures we were also able to correctly classify the LD children with a precision of > 90%. Thus, these EEG techniques might be useful for objective classification and diagnosis of LD children, which is particularly important for non-Western countries. In addition, these atypical neurophysiological activation patterns might provide a helpful guide for rehabilitation strategies to treat the deficiencies in LD children.

Disclosures: **N. Alahmadi:** None. **L. Jancke:** None.

Poster

585. ADHD and Other Behavior Disorders

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CASS foundation

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Australian Postgraduate Awards

Title: Dysregulation of SRY in the male brain: a genetic basis for male susceptibility to neurological disorders?

Authors: *H. P. LOKE[1,2], P. PINARES-GARCIA[1,3], V. HARLEY[1,2,3], J. LEE[1,2,3];
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Abstract: Sex differences in the dopamine (DA) pathway are likely to underlie male susceptibility to neurological disorders such as Parkinson's disease (PD) and attention deficit hyperactivity disorder (ADHD). Aside from hormonal effects, recent evidence suggests that sex-chromosome genes may influence the sex differences in the healthy and diseased DA system. The Y-chromosome gene, *SRY*, is an ideal candidate to study, as it is found in various DA abundant brain regions. Our previous *in vivo* studies showed that reducing *SRY* expression in midbrain cells significantly reduced motor function and expression of DA machinery genes in male rats, suggesting that SRY regulates DA biosynthesis and motor function in males. Thus, we propose that dysregulation in SRY expression could underlie male susceptibility to DA associated disorders such as PD and ADHD. To test this hypothesis, we investigated the regulation of *SRY* in the normal and diseased male DA system using *in vitro* and *in vivo models* of PD and ADHD. In the human male dopaminergic cell line, M17, *SRY* expression was significantly increased at 1h following DA treatment with no significant changes in *GADD45γ* (a marker of DNA damage and a known regulator of *SRY*) expression and cell viability, indicating that *SRY* is regulated by a *GADD45γ*-independent (possible DA receptor-dependent) mechanism. However, increase in *SRY* expression at 6 and 12h was associated with significant increases in *GADD45γ* expression and increased cell death. Similarly, treatment with the DA toxins 6-hydroxydopamine (6-OHDA) or rotenone increased *SRY* and *GADD45γ* expression at 6 and 24h. In the 6-OHDA lesioned rat model, unilateral injection of 6-OHDA increased nigral *SRY* and *GADD45γ* expression from days 2 to 14 post-injection, which was associated with reductions in limb use and nigral *TH* expression in male rats. Injection of rotenone, also elevated *SRY* and *GADD45γ* expression at day 7 post-injection. In the spontaneously hypertensive rats (SHR), a well charaterised animal model of ADHD, male SHR showed significant hyperactivity in velocity and distance travelled as compared to the control male Wistar Kyoto rats (WKY) in the open field test. These behavioral differences were associated with significantly lower *SRY* expressions in the prefrontal cortex, hippocampus, hypothalamus and substantia nigra of male SHR compared to male WKY. In conclusion, we showed that SRY is upregulated *in vitro* and *in vivo* models of PD, and downregulated in a ADHD model, indicating that *SRY* dysregulation may underlie male susceptibility to these disorders. Thus, normalizing *SRY* expression may be a potential therapeutic strategy for treating DA-associated disorders such as PD and ADHD.

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Poster

585. ADHD and Other Behavior Disorders

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Topic: A.07. Developmental Disorders

Support: ANR Samenta 015 02

Federation pour la Recherche sur le Cerveau

University Lyon 1

Title: Tool-induced learning and plasticity in motor control of healthy and DCD teens

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Abstract: Body Schema plasticity has recently gained increasing interest, as the update of our body-parts posture and length might be the basis for humans' exquisite mastery of tools. To efficiently control our actions, whether performed with our own body effectors or with tools, the brain needs to store and update all the metrics of the body (from the coding of initial hand position, to the evolving state of the effector, through the dimensions of the body). Previously (Baccarini et al, 2014; Cardinali et al, 2009, 2011, 2012, 2016), we reported that using a tool functionally extending the arm leads to a fast plastic update of the arm representation for action, namely the Body Schema (BS). Tool-induced modifications are reliably observable on the kinematics of subsequent free-hand movements.
Here, our aim was twofold. First, we examined whether this fast body plasticity co-exists with the slow body metrics modifications induced by growth. Second, we investigated body representations in adolescents affected by Developmental Coordination Disorder (DCD) as inaccurate body representations might lead to general clumsiness, or poor motor skills.
We assessed the kinematics of typical developing and DCD adolescents in a task requiring to reach for an object before and after having performed the same action with a tool. In addition, we examined their imitation skills using the De Renzi scale for apraxia. Their pubertal development was evaluated through a Pubertal Development Scale.
Results indicate that tool-induced plasticity develops slowly, becoming apparent only after puberty that is when an adult body dimension has been reached. Indeed, the more mature were adolescents, the more they displayed, as observed in adults, peaks of longer latencies and smaller amplitudes after tool-use. DCD adolescents did not display the typical tool-induced plasticity as they performed their movements faster after tool-use (reduced latencies, increased peaks),

kinematics values reaching a normal range.
While the body representation for action undergoes fast updates in adults, its plasticity does not seem to co-exist with the slow update required by growth. This lack of BS fast plasticity might be linked to the clumsiness described in teenagers, as further suggested by their difficulties in imitating arm and finger gestures and postures. Adolescents with DCD did not update their arm representation either, interestingly though, their free-hand movements tended to normalize after tool-use.

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Poster

585. ADHD and Other Behavior Disorders

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Topic: A.07. Developmental Disorders

Support: NIH Grant P50 HD40095, R01 HD056107

NIH Grant T32 NS041218

Title: An fMRI and fcMRI study of the motor system in children with and without dyslexia

Authors: ***T. TURESKY**[1], O. OLULADE[2], G. EDEN[2];
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Abstract: The defining feature of developmental dyslexia is difficulty in learning to read. However, motor impairments are also reported in studies of dyslexia (Wolff, 2002) and there are several theories that attempt to account for these observations. The cerebellar deficit hypothesis proposes that reading difficulties result from dysfunction in motor-articulatory systems that relate to phoneme processing and/or automatization of motor and other skills (Nicolson et al., 2001). Differences in activity in the cerebellum have been observed in adults with dyslexia during sequential finger pressing of learned and novel sequence (Nicolson et al., 1999) and serial reaction time tasks (Menghini et al., 2006). The latter also identified differences in supplementary motor area (SMA), right lateral premotor cortex, and inferior parietal cortex. It is unknown, however, if these differences also present during simple, externally paced finger movements commonly used in studies of the motor system (Witt et al., 2008). To address this question fMRI data was acquired in 17 dyslexic (10.2 ± 1.4 years; Woodcock-Johnson III (WJ III) Word ID < 92) and 16 non-impaired (9.6 ± 3.2 years; Word ID > 92) right-handed children

while performing irregularly paced, separate left and right thumb tapping tasks. Whole-brain fMRI and functional connectivity (fcMRI) analyses were performed within and between groups using SPM12 and CONN 16a with in-scanner head motion and reaction times entered as nuisance covariates. Seed-to-voxel correlations were used for fcMRI with 6 mm spheres in ipsilateral cerebellum, contralateral primary sensorimotor cortex (SM1), and SMA. Clusters of activity (height threshold $p = 0.001$, a cluster-level FDR correction at $p < 0.05$) were found in children with and without dyslexia in contralateral SM1, bilateral SMA and ipsilateral cerebellum for movements of the thumb with either hand. Between-group comparisons during right finger tapping revealed greater activation for dyslexic compared to non-impaired readers in a cluster (MNI=6, -27, 62) containing bilateral paracentral lobule (BA 5) and extending into SMA and precentral gyri (BA 6). Activity here was correlated with WJ III Reading Fluency ($r = -.48$; $p < .005$) and Rapid Automatized Naming (RAN) time ($r = -.48$; $p < .005$). FcMRI was observed for each group, but no between-group differences were found. In conclusion, there was no evidence of cerebellar dysfunction in dyslexia, but instead aberration of cortical motor function underlying right finger movement.

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Poster

585. ADHD and Other Behavior Disorders

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Topic: A.07. Developmental Disorders

Support: NIH Grant 1-ZIA-MH002860-08

Title: Reduced amygdala response to fearful expressions in youth with callous-unemotional traits related to faster habituation

Authors: ***H. MEFFERT**[1], P. M. TYLER[1], M. L. BOTKIN[1], A. K. ERWAY[1], V. KOLLI[1], S. F. WHITE[1], K. POPE[1], J. R. BLAIR[2];
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Abstract: Youth who display increased levels of externalizing behavior and whom additionally present with elevated callous-unemotional (CU) traits (lack of empathy and remorse/ guilt) show reduced sensitivity to other peoples' fear. This is accompanied by reduced amygdala responsiveness. However, the amygdala has also been shown to habituate to repeated stimulation by facial expressions, and therefore, the reduced average responsiveness to fearful facial

expressions might be related to stronger habituation to these stimuli in youth with elevated CU traits. In the current study, we included 77 youth with varying levels of externalizing behavior, who performed a gender discrimination task on photographs of actors displaying fearful expressions of increasing intensity. The task was split into two runs. Analyzes were conducted within the framework of the Research Domain Criteria project, meaning that a dimensional approach was adopted. The inverse relationship between amygdala responsiveness and level of CU traits was significantly greater in run 2 relative to run 1. In addition, connectivity between the amygdala and the anterior insula, posterior cingulate cortex and several regions in the visual cortex was significantly more inversely related to the participant's level of CU traits during run 2, compared to run 1. These data suggest that previous findings of generally reduced average responsiveness to fearful facial stimuli in youth with elevated CU traits might reflect greater habituation to these stimuli in these youth.

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Poster

585. ADHD and Other Behavior Disorders

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Topic: A.07. Developmental Disorders

Support: NIH/NIAAA Training Grant P50AA022534-01, T32 AA014127

Title: Joint independent component analysis of brain structure and function in adolescents with FASD and healthy controls

Authors: *J. F. PINNER[1,2], B. A. COFFMAN[3], A. D. BOLANOS[1], P. W. KODITUWAKKU[4], J. M. STEPHEN[1];
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Abstract: With an estimated incidence rate of 1/100 live births, Fetal Alcohol Spectrum Disorders (FASD) present a unique minutiae of clinical and social implications and difficulties. As children with FASD share similar behavioral profiles with other disorders such as attention deficit hyperactivity disorder (ADHD) and oppositional defiant disorder (ODD), while simultaneously being underreported throughout society, identifying reliable neurophysiological markers is paramount to help us understand this widespread disorder, and to develop appropriate

treatment interventions. Previous studies have identified both structural and functional deficits associated with FASD, yet it is unclear how these structural deficits directly impact function. To gain further insight into the combined impact of structural and functional deficits we employed a multivariate joint independent component analysis (jICA) to identify linked components that covaried across adolescents (age 12-21) with FASD (n=11) and healthy controls (HC: n=17). We focused on measures of Fractional Anisotropy (FA) and neural responses to sensory stimuli as measured with Magnetoencephalography (MEG) using bilateral auditory (Aud), somatosensory (Som), and Visual (Vis) stimuli. We found 5 joint components representing a pattern of covariation across FA and sensory evoked responses that showed significant group differences. The first component (p=0.023) indicated that HC showed a stronger association than FASD between FA in cerebellar white matter tracts and MEG amplitude in the R temporal, R central, and L and R occipital regions (MEG) during Aud, Som, and Vis stimuli. These findings provide evidence for a correspondence between localized alterations in structural connectivity (FA) and functional responses (MEG) in FASD. Considering the majority of individuals with FASD exhibit no identifiable morphological alterations compared to HC, identifying markers specific to FASD is essential to avoid misdiagnosis and to inform early life interventions.

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Poster

585. ADHD and Other Behavior Disorders

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Topic: A.07. Developmental Disorders

Support: PAPIIT (proyecto IN204613)

CONACYT (251309)

Title: Arithmetic processing in children with dyscalculia. An event-related potential study.

Authors: *C. S. SONIA YANIN, ESQ[1], T. FERNANDEZ[2], J. SILVA[3], B. PRIETO-CORONA[3], M. ROCA-STAPPUNG[2], G. ALATORRE-CRUZ[3];
[1]Inst. de Neurobiologia, [2]Neurobio. Inst., UNAM, Queretaro, Mexico; [3]Psicology, UNAM-Iztacala, Estado de Mexico, Mexico

Abstract: Dyscalculia (Dy) affects academic performance and daily life, a characteristic of this disorder is the difficulty to identify if the result of an arithmetic operation is correct or not.

Studies of Event-Related Potential (ERP) report that, unlike children with Dy, normal children show N400 and P600 components in the incorrect probe of an arithmetic operation. Objective: compare electrophyisiological activity during arithmetic processing between a group of 20 children with Dy and a group of 18 children with normal academic performance (NAP). We recorded ERP during an arithmetic verification task. Each trial corresponds to one-digit sum followed by a correct or incorrect probe (experimental conditions). Children decided if their own result match or mismatch regarding the probe. ERP were obtained time-locked to onset of the probe. A non-parametric permutation multivariate analysis was applied to evaluate differences between conditions. In both groups there was a higher percentage of right answers for the incorrect-probe compared to correct-probe. In both conditions, the percentage of right answers was higher in NAP than Dy group. NAP group showed an N400 effect (higher amplitudes to incorrect-probes than correct-probes) and a late positive component with widespread distribution (higher amplitudes to correct-probes than incorrect-probes). In contrast, Dy group displayed no significant N400 effect but a focal (P4, O1, O2, T6) late positive component. This suggests that Dy group has significant problems from arithmetic and matching-probe processes, reflected by N400, to a deficient verification process manifested by the late positivity. Acknowledgments: Héctor Belmont, María Elena Juárez, Héctor Belmont, PAPIIT (proyecto IN204613) & CONACYT (251309).

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Poster

585. ADHD and Other Behavior Disorders

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Topic: A.07. Developmental Disorders

Support: Children's Mercy Hospital

NIH Grant HD02528

Title: Cerebellar anatomical and neurochemical dysfunction with hyperbilirubinemia in 21-day-old and 4-month-old Gunn rats

Authors: *J. A. STANFORD[1], D. MA[3], J. L. HARRIS[2], B. SNYDER[1], F.-C. YANG[1], W. M. BROOKS[2], S. M. SHAPIRO[4];
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City, KS; [3]Huazhong Univ. of Sci. and Technol., Wuhan, China; [4]Pediatrics, Children's Mercy Hosp., Kansas City, MO

Abstract: Bilirubin-induced neurological dysfunction (BIND) is implicated in a wide range of neurodevelopmental disorders. In jaundiced Gunn rats (jjs) hyperbilirubinemia (HB) peaks between 16-21days of age, and jjs exhibit hyperactivity and gait abnormalities implicating cerebellar dysfunction. Determining cerebellar effects of HB at the time of high exposure and subsequently may provide biomarkers to diagnose BIND and new targets for intervention. In order to do this, we used ^{1}H-MRS in a 9.4T MRI to quantify neurochemicals related to oxidative stress, astroglial activation, inflammation, excitatory neurotransmission, bioenergetics, and neuronal integrity in the cerebellum of jjs and their non-jaundiced (Nj) littermates at 21days and 4 mos of age. We also measured body weight, total plasma bilirubin, locomotor activity, and MRI cerebellar volume. At 21 days bilirubin levels in jjs were 13.5±1.1 mg/dL and 13.3±0.4 mg/dL in jjs that were in the 21 days and 4 mos imaging groups, respectively. At 21 days jjs exhibited significant increases in neurochemicals related to glutamate turnover (glutamine and glutamine/glutamate ratio), excitotoxicity (serine), astroglial activation/inflammation (myo-inositol), oxidative stress (ascorbate, glutathione), cell membrane turnover and breakdown (glycerophosphocholine, phosphocholine), and significant decreases in bioenergetic function (aspartate), and neuronal integrity (N-acetylaspartate, taurine). At 4 months markers of decreased neuronal integrity were still present, and glutamate and macromolecules were decreased in jj rats, but the other markers were no longer different between jjs and Njs. Cerebellar volume was significantly decreased in jjs at both ages, and in 21 day jj rats, cerebellar glucose was significantly correlated positively with myo-inositol (r=0.86) and negatively with body weight (r=-.76) and cerebellar volume (r=-.77). In summary, we found abnormal neurochemical events in the cerebellum of jj rats. The transitory effects at the time of peak HB, including the potential relationships between glucose and measures of astroglial activation/inflammation, oxidative stress, cerebellar volume, and body weight, provide therapeutic targets for intervention in BIND, and long-term abnormalities suggest possible ^{1}H-MRS biomarkers in older children and/or adults to implicate BIND.

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Poster

585. ADHD and Other Behavior Disorders

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Topic: A.07. Developmental Disorders

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College of Education Seed Grant, Boise State University

Think Tank Grant, Lafayette College

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Title: Early detection of reading impairment with virtual maze environment: a longitudinal study

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Abstract: Reading disorder (RD, a.k.a. Dyslexia) is a specific learning disability that affects reading. If learning disabilities remain untreated, a child may experience long term social and emotional problems which influence future success in all aspects of their lives. Early detection and intervention will help to close the gap between typically developing and reading impaired children in acquiring reading skills. Although our understanding and treatment for dyslexia has greatly increased in the last 20 years, a significant percentage of children with dyslexia are either identified too late, or have a specific manifestation of the disorder that is not understood well enough to design and deliver a successful remediation. Research examining the connection among genetic, cognitive and behavioral aspects of reading disorder offers promise for early identification and intervention to successfully address specific phenotypes of RD. Recent research from our laboratory demonstrates that 8-13 year olds with RD exhibit impaired performance on a virtual Hebb-Williams (vHW) maze task. In this study we examined performance on the vHW maze in 5-6 year old children who identified as either typical or struggling readers. This virtual maze test does not rely on text or require rapid access to phonological processing; therefore was not influenced by a potential difference in reading experience between groups. The goal of this study was to determine if pre-readers at risk for RD, exhibit altered visuo-spatial processing on the vHW maze task and risk variants of candidate dyslexia susceptibility genes (CDSGs). Our data demonstrate that struggling readers are impaired at the vHW maze task and performance on this task correlates with specific CDSG risk variants. Since the predictive validity of the early reading measures with later reading ability vary from as low as .38 to as high as .74, children who present as at risk at a young age may develop strong reading skills over time, and vice versa. In addition, sensory impairments can change over the course of development (i.e. experience) leading to a change in behavior. Therefore the 5-6 year old children were reexamined at ages 8-9 for reading and vHW maze performance. These studies are essential in order to understand the link between CDSG risk variants and cognitive processing deficits reported in individuals with dyslexia so that more comprehensive interventions can be developed to reach students with RD currently labeled as ‘non-responders’ to conventional methods. This is an important first step to early detection of RD, which may lead to a significant decrease in the delayed acquisition of reading skills.

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Poster

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Topic: A.07. Developmental Disorders

Support: Swedish Research Council

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Title: Studies of the molecular pathways behind dyslexia

Authors: ***A. BIEDER**[1], H. MATSSON[1], A. FALK[2], J. KERE[1], I. TAPIA-PÁEZ[1];
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Abstract: Dyslexia is a complex learning disability characterized by deficits in reading despite adequate intelligence, normal senses and proper socio-cultural opportunities. It is the most common reading disorder, affecting 5-10% of the population. Dyslexia has a strong genetic component and many susceptibility genes have been identified among which *DYX1C1*, *DCDC2* and *KIAA0319* are the most replicated. The molecular functions of these genes are little investigated, yet all the above have been implicated in neuronal migration and development. More recently, our group and others have shown a link between dyslexia candidate genes and cilia. We are investigating the role of dyslexia candidate genes in relation to cilia and centrosome.We are thus performing assays in a human ciliated cell line (RPE1, retinal pigment epithelial cells) and in tissues for sub-cellular localization by immunofluorescence (IF). In addition, we are looking for common molecular pathways of the candidate genes *DYX1C1*, *DCDC2* and *KIAA0319* by functional assays. We are using neuroepithelial-like stem cells (NES cells) derived from induced pluripotent stem cells (iPSCs) as a model to further dissect the molecular mechanisms involved in dyslexia. NES cells are self-renewing and can be differentiated along the neural and glial lineages. We have shown by qRT-PCR and IF that *DYX1C1*, *DCDC2* and *KIAA0319* gene expression is upregulated in NES cells during their

differentiation to neurons and glia. In addition, they are growing cilia during differentiation, as shown by IF and qRT-PCR of ciliary markers. NES cells are thus a valid model system to study the function of the dyslexia candidate genes and their role in cilia. We observe a localization of DCDC2 in the axoneme of some neuronal cilia upon differentiation and a localization of DYX1C1 around the basal body. Interestingly, CPAP/CENPJ - a centrosomal protein with a role in the regulation of microtubule assembly and disrupted in microcephaly - interacts and co-localizes with DYX1C1 at the centrosome. In a parallel approach, we are using lentiviral expression constructs to overexpress *DYX1C1*, *DCDC2* and *KIAA0319* in NES cells in order to study the downstream pathways of these genes.

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Poster

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ACT1113

Title: Acute and chronic treatment of methylphenidate improve and impair the sinaptic plasticity by change in the insertion of AMPA-GluA1 receptor subunits in pyramidal neurons of the hippocampal CA1 area

Authors: *C. A. CARVALLO[1], D. CONTRERAS[2], R. FARIAS[2], D. MORALES[2], G. UGARTE[2], R. DELGADO[2], C. ROZAS[2], M. ZEISE[3], B. MORALES[2];
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Abstract: Methylphenidate (MPH) is widely used in the treatment for Attention Deficit Hyperactivity Disorder and recently as a drug of abuse. It is known that acute exposure of MPH improve and chronic administration impairs the long term memory. However, the cellular and molecular and mechanisms involved in these synaptic processes are poorly understood. We address this question studying the visuo-spatial memory (VSM), hippocampal long term potentiation (LTP) and insertion of AMPA receptors in the plasma membrane in both conditions. Six sessions in the Morris Water Maze (MWM) were used to evaluate the acute and chronic effects on the VSM; rats p21 were administered i.p with a single dose of 1 mg/kg MPH or repeated dose during 6 days, respectively. Hippocampal slices of 400 μm of thickness were obtained of the same Sprague-Dawley rats used in the behavior test. LTPs were induced by theta burst stimulation (TBS) applied in Schaeffer collateral and recording field excitatory postsynaptic potentials in the Striatum radiatum of CA1. Finally, the slices were processed to evaluate the changes of surface-associated AMPA receptor employing western blot and crosslinking assays. Control and rats treated with single dose of MPH reduced the time to reach the platform in consecutive trials of MWM. A significant decrease was found in the 2th session (Control: 83.3±5.7 s; MPH: 58.8±7.3 s; $p<0.05$; n=21). In contrast, a significant increase was observed during the 2th (54.1±8.9 to 88.9±7.3 s; $p<0.05$; n=21) and 4^{th} (33.4±5.9 to 62.9±9.8 s) sessions between the controls and chronically treated rats (n=21, $p<0.05$). To study the electrophysiological correlate of behavioral studies, we induce LTP in slices from the same animals used in behavioral studies. A significant increase from 150.1±0.2% to 179.9±0.3% (n=4,7; $p<0.001$) was observed in the TBS-dependent LTP in acute conditions, in contrast, a significant decrease from 156.14±0,3% to 131.19± 0.5% (n=4,6; $p<0.001$) was observed in chronical conditions. To investigate the molecular mechanisms we evaluate the insertion of new AMPA receptors in the surface. Consistent with our electrophysiological results, we found a significant increase in the protein levels of GluA1 subunit from 0.46±0.02 to 0.73±0.02 (n=4, $p<0.05$) in acute conditions and a significant decrease from 0.45±0.01 to 0.29±0.02 (n=4, $p<0.05$). These results suggest that acute and chronic applications of MPH induce a differential effect on the plasticity. MPH improved the behavior performance enhancing LTP by insertions of AMPA-GluA1 receptor subunits and impaired the behavior performance decreasing the LTP due to removing of AMPA-GluA1 receptor subunits in plasma membrane.

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Poster

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Topic: A.07. Developmental Disorders

Support: Ellison Medical Foundation Grant

Title: Altered brain bases of rapid naming in adults with dyslexia

Authors: *T. M. CENTANNI[1], S. N. DEL TUFO[2,1,3], J. D. E. GABRIELI[1], J. A. CHRISTODOULOU[1,4];
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Abstract: Performance on rapid automatized naming (RAN; the ability to automatically and efficiently retrieve labels for abstract visual stimuli) predicts reading ability and relates to reading fluency. RAN performance is often impaired in readers with dyslexia but no study has yet compared the difference in brain activation during RAN performance between typical reading and struggling reading groups. Using letter, number, and alternating alphanumeric stimuli, we compared brain activations for adults with dyslexia to an age-matched sample of typically reading individuals. To evaluate developmental differences of brain activations during this task, we also included a sample of typically reading adolescents. As expected, adults with dyslexia and typically reading adolescents were significantly slower at performing all conditions of the in-scanner RAN task (letters, numbers, and alternating alphanumeric stimuli) compared to typical adults. Typically reading adults recruited posterior networks significantly more than adults with dyslexia during tasks containing letter stimuli (letter and 2set naming). Compared to typical adult and adolescent readers, adults with dyslexia exhibited more activation in right frontal and parietal areas during RAN letter tasks, while RAN number tasks evoked more activation in bilateral frontal and several right hemisphere areas in both adult groups compared to the typical adolescent group. Activation during RAN letter naming in the posterior occipital brain regions not only correlated with performance across groups on several word-reading measures, but was also correlated with rapid naming speed for all stimulus conditions. These results support previous hypotheses that the system for rapid naming overlaps with the reading brain system, and this differs between reader groups. Specifically, rapid naming activates the reading network of the brain, and activation patterns are comparable in reading and RAN in individuals with dyslexia. Implications for understanding the neural system associated with reading and component skills are discussed.

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Poster

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DFG Grant STR 597/7-1

IZKF Junior Research Group 2115-0-0

Title: Predictors of treatment outcome for neurofeedback trainings in adult attention deficit hyperactivity disorder (ADHD)

Authors: *A.-C. EHLIS[1], B. BARTH[1], K. WITTKE-LAUBE[2], K. MAYER[3], U. STREHL[3], A. J. FALLGATTER[1];
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Abstract: Over the last decades, neurofeedback trainings have been established as a complementary neurobiological intervention in attention deficit hyperactivity disorder (ADHD). Even though meta analyses in childhood populations indicate clinical improvements over the course of neurofeedback interventions with typically medium effect sizes, the specificity of the training effects is discussed controversially. In the present study, 60 adult patients with ADHD were randomly assigned to 30 sessions of either a conventional EEG-based neurofeedback protocol (slow cortical potential / SCP training), a newly developed neurofeedback training based on near-infrared spectroscopy (NIRS) and focusing on frontal lobe function, or an electromyography-based biofeedback training (control group). Linear regression analyses were applied to identify predictors of treatment outcome in terms of changes in hyperactivity, impulsivity and inattention scores (self-report measure). For the overall sample, comorbid depressive and borderline symptoms at baseline as well as patient expectations regarding the success of the treatment were tested as potential (unspecific) predictors of the training outcome. In subgroup analyses, we furthermore tested the hypothesis that a particularly impaired frontal lobe function at the beginning of the training (as indicated by frontal lobe activation during NoGo trials of a Go/NoGo task) would predict a particularly good treatment outcome specifically within the NIRS frontal lobe training group. Preliminary data analyses show 1) significant symptom improvements in all three trainings groups, with some indications of a superiority of the NIRS training regarding impulsivity scores; 2) no significant prediction of treatment outcome by comorbid symptoms or patient expectations; and 3) a significant

differential prediction of treatment outcome by initial frontal lobe function in both the NIRS and EEG training group. In more detail, a particularly weak initial frontal lobe function was associated with particularly pronounced improvements in impulsivity scores in the NIRS training group, whereas in the SCP group, a particularly strong frontal lobe function at baseline predicted the best training outcome (inattention and hyperactivity scores). The results indicate that an assessment of frontal lobe function before the beginning of a neurofeedback training might be a useful tool to assign adult ADHD patients to either an SCP (EEG) or frontal lobe training (NIRS), which might contribute to a more individualized treatment and optimize the effects of neurofeedback interventions.

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Poster

585. ADHD and Other Behavior Disorders

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Topic: A.07. Developmental Disorders

Support: SFB 779 (TP A03)

Title: Altered anticipatory reward representation in the orbitofrontal cortex in children and adolescents with ADHD

Authors: *J. TEGELBECKERS[1], M. KANOWSKI[2], C. BREITLING[1], K. KRAUEL[1,3], J.-D. HAYNES[4,5,6], H.-H. FLECHTNER[1], T. KAHNT[7];
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Abstract: Alterations in reinforcement sensitivity are a well-established finding in patients suffering from attention deficit hyperactivity disorder (ADHD) and are thought to depend on dopaminergic dysfunctions in the striatum (Luman, 2010). However, in addition to dopaminergic activity, reinforcement learning also depends on reward representations in the orbitofrontal cortex (OFC; Kahnt, 2010). It is unclear whether OFC reward representations differ in ADHD

patients as well.
In the current study, 16 ADHD patients and 18 typically developing (TD) children and adolescents (11-16 years) performed a delayed perceptual decision-making task in which the orientation or color of triangles had to be reported. The combination of both visual features indicated whether a high or a low reward was provided for a correct response. To obtain high-resolution measures from the OFC despite reduced BOLD sensitivity in this region, we developed an optimized BOLD sequence (partial volume, 24 slices, voxel size = 1.25x1.25x 1.8 mm^3, TE=30ms) allowing complete coverage of the OFC with improved sensitivity.
We found that both groups showed similar activation patterns during receipt of reward in several brain areas, including the bilateral OFC, insula and parahippocampus. However, during anticipation of high rewards, compared to TD, children and adolescents with ADHD displayed significantly stronger activity in the bilateral OFC as well as parahippocampus, left amygdala and left anterior cingulate.
Our results suggest that while OFC representations for received reward value are generally intact in children and adolescents with ADHD, patients show enhanced OFC activity for large expected rewards, suggesting a higher sensitivity to anticipated reward value in the disorder.
Luman, M. (2010). Identifying the neurobiology of altered reinforcement sensitivity in ADHD: a review and research agenda. Neurosci. Biobehav. Rev., 34(5), 744-754.
Kahnt, T. (2010). The neural code of reward anticipation in human orbitofrontal cortex. PNAS, 107(13), 6010-6015.

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Poster

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Topic: A.07. Developmental Disorders

Support: NIH training grant T32 007051

Title: Developmental deltamethrin exposure alters adult learning and behavior

Authors: *E. M. PITZER[1], M. T. WILLIAMS[1,2], C. V. VORHEES[1,2];
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Abstract: Attention-deficit hyperactivity disorder (ADHD) is estimated to affect 8-12% of school age children. It has been suggested that environmental factors may contribute to the

etiology of ADHD. In this regard, pesticides have been suspected. We exposed Sprague-Dawley rat pups to the Type II pyrethroid deltamethrin (DLM) at doses of 0, 0.25, 0.5, and 1.0 mg/kg from postnatal day 3-20. DLM increased mortality at the 1 mg/kg dose and reduced body weight at 0.5 and 1 mg/kg. In the Morris water maze (MWM) on acquisition for path efficiency there was a significant treatment main effect ($P<0.04$) whereas on reversal the effect fell short of significance ($P=0.066$). This pattern was similar for latency and path length on both acquisition and reversal. There was also a trend for a treatment effect on conditioned fear ($P=0.0725$). In the open-field there was a treatment x interval interaction ($P<0.02$) that by slice-effect ANOVA was in intervals 1 and 7 in which DLM groups were less active than Controls (recorded in 5-min intervals for 1 h). For prepulse-inhibition (PPI) of acoustic startle, there were significant treatment x sex ($P< 0.01$) and Treatment x Sex x PPI ($P<0.03$) interactions. Further analyses showed the effects to be in females ($P<0.01$). Among females, the effects were on the no-prepulse ($P<0.003$) and low-intensity prepulse trials ($P<0.002$). In both cases the differences were between the 0.5 and 1 mg/kg DLM groups vs. Controls ($Ps<0.01$). On these trials, DLM dose-dependently reduced startle amplitude (V_{max}). PPI assesses sensorimotor gating, an attention-related process that may relate to attentional aspects of ADHD. More litters are being tested. The data indicate that neonatal DLM exposure has adverse long-term effects on learning and attention, effects consistent with an ADHD-like phenotype.

Disclosures: E.M. Pitzer: None. **M.T. Williams:** None. **C.V. Vorhees:** None.

Poster

585. ADHD and Other Behavior Disorders

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Topic: A.07. Developmental Disorders

Title: A potential animal model for the hyperactive phenotype of ADHD

Authors: *I. I. DELA PENA[1], J. DE LA PENA[1], H. KIM[1], I. DELA PENA[2], J. RYU[3], B.-N. KIM[4], D. HAN[5], J. CHEONG[1];
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Abstract: Attention-deficit/hyperactivity disorder (ADHD) is the most common neurodevelopmental disorder affecting ~ 5% to 10% of children worldwide. It characterized by

the three core symptoms of hyperactivity, impulsivity, and inattention. To date, the exact cause of ADHD is still unknown, but substantial evidence has shown that this disorder has a significant genetic component. Genetic animal models have been highly valuable in understanding this disorder. Though it cannot exactly reflect the human condition, animal models can give understanding into the disorder that cannot be achieved from human subjects because of various limitations. Due to its complexity (heterogeneous disorder), a sole model is unlikely to be able to simulate all of its symptoms. Nonetheless, a model that recapitulates key endophenotypes can be beneficial in understanding its etiology and providing specific therapeutic implications. Thus, the present study sought to develop an animal model of ADHD which would recapitulate the hyperactive phenotype of the disorder. Towards this, we studied common differentially expressed genes (DEGs) in the prefrontal cortex of SHR/Ncrl, the most validated animal model of ADHD. SHR shows hyperactivity in the open-field test, as compared to Wistar and WKY rats, a strain representing the "normal" heterogeneous population. Furthermore, hyperactivity in SHR was attenuated by treatment of psychostimulants. The common DEGs in the PFC of SHR vs. WKY/NCrl and Wistar rats are those involved in transcription, circadian rhythm, and corticotropin secretion. We then made transgenic animal models overexpressing one of these genes. Here we report our initial finding that mice overexpressing one of the candidate genes indeed showed hyperactive behavior. Additional studies are underway to further characterize the behavior of this new transgenic animal model and to exemplify any underlying neurobiological mechanisms.

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Poster

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Topic: A.07. Developmental Disorders

Title: Age effect on brain functional network graph metrics in patients with attention deficit hyperactivity disorder and normal children and adolescents.

Authors: *B. MOHAJER, N. ABBASI, A. ABDOLALIZADEH, N. HOSSEINI;
Student Scientific Res. Ctr. of Tehran Unive, Tehran, Iran, Islamic Republic of

Abstract: Introduction: attention deficit hyperactivity disorder (ADHD), as one of the most common childhood and adolescent's neuropsychiatric disorders, has shown to demonstrate impairments of structural and functional neural connectivity networks, a finding that occurs in

the maturation and pruning period of the brain synapses. Comparing effect of ageing on brain network development between healthy children and adolescents and ADHD patients would help to clarify this process. **Methods:** We used resting-state functional correlation matrices of 190 ADHD patients and 330 age and sex matched healthy children and adolescents from ADHD200 study (umcd.humanconnectomeproject.org). Minimum, maximum and average ages were 7.17, 21.74 and 11.83 years respectively. Each matrix consisted of undirected weighted correlations between 200 regions in fMRI of subjects (Craddock atlas). Graph theoretical analyses were performed using the Brain Connectivity toolbox. In order to enable comparison of global network properties across groups we applied 20% sparsity threshold based on previous studies. Global efficiency, Transitivity, Assortativity and Modularity were calculated as global network measures. Using R for statistical analyses, measures were fed into general Linear Model (GLM), to investigate the age effect controlled for gender effect. **Results**: We found significant decrease in Assortativity with increase of age in healthy subjects after FDR correction (P value=0.018, t-value=-2.3) compared to non-significant result with slower slop of asoortativity decrease in ADHD (P value=0.19, t value=-1.3) patients. The remaining measures did not reveal any significant changes with increase of age. **Discussion:** Number of neighboring nodes that sharing an edge with a node, defines as the degree. Assortativity indicates how preferentially nodes of similar degree connect to each other. As previously shown, adult and child functional brain networks both demonstrate small-world features, although consisting of different communities of nodes. The difference here is nodes arrangement: children's communities arranged by anatomical proximity on the other hands adults' predominantly reflect functional relationships. Above results may indicate architectural changes in functional network of normal population, from anatomical proximity connectivity through functional relationships. This connectivity pattern shift may be the main reason behind neural network malfunctions in ADHD, what reflects in amount of assortativity. Future functional connectivity studies focusing on the regional measures may clarify network dynamics in ADHD population.

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Poster

585. ADHD and Other Behavior Disorders

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Support: DFG SFB 779, TP A03

NIH Grant P41 GM103545-17

Title: Optimized stimulation protocol of the rIFG for the application of tDCS in children and adolescents with ADHD

Authors: *C. BREITLING[1], K. KRAUEL[1,4], T. ZAEHLE[2], J. TEGELBECKERS[1], H.-H. FLECHTNER[1], V. LESSMANN[3,4], C. SEIDENBECHER[5,4], S. GULER[6,7], M. DANNHAUER[6]; [1]Child- and Adolescent Psychiatry, [2]Neurol., [3]Inst. of Physiol., Otto-von-Guericke Univ., Magdeburg, Germany; [4]Ctr. for Behavioral Brain Sci., Magdeburg, Germany; [5]Dept. of Neurochemistry and Mol. Biol. and Dept. of Behavioral Neurol., Leibniz Inst. for Neurobio., Magdeburg, Germany; [6]Scientific Computing and Imaging Institute, Ctr. for Integrated Biomed. Computing, Univ. of Utah, Salt Lake City, UT; [7]Dept. of Electrical and Computer Enginieering, Northeastern Univ., Boston, MA

Abstract: Transcranial direct current stimulation (tDCS) is a non-invasive method that can induce changes in intracortical excitability, plasticity and neuronal activity and its use in patients with ADHD has been suggested as a promising alternative to psychopharmacological treatment approaches. Results of a prior investigation (Breitling et al., 2016, Front. Cell. Neurosci.) suggest that anodal tDCS of the right inferior frontal gyrus (rIFG) could improve interference control in adolescents with ADHD but results were inconsistent between patients. However, a simulation of the induced current flow revealed a diffuse current densitiy distribution in widespread brain areas due to the stimulation. For future applications we wanted to increase precision and effectiveness of the setting. A method with enhanced spatial focality that induces maximum current flow directly under the stimulation electrode uses a 4x1 ring electrode configuration that, in contrast to conventional tDCS, restricts the current flow to the area under the electrodes. The software "SCIRun5/BrainStimulator" and a multimodal child head model were used to compute an optimized electrode configuration for the stimulation of the rIFG in children and adolescents. We found that the optimized montage induces more current flow in the rIFG than conventional tDCS while diffuse current flow in the rest of the brain was reduced. To provide improved controllability of stimulation is of special importance in children and adolescents in order to avoid side effects. Currently, data are collected in children and adolescents with ADHD to investigate whether optimized stimulation of the rIFG yields indeed superior results.

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Poster

585. ADHD and Other Behavior Disorders

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Topic: A.07. Developmental Disorders

Support: Supported by Jim and Betty Ann Rodgers Chair Fund

Title: Perinatal nicotine exposure mouse of attention deficit hyperactivity disorder

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Abstract: Attention deficit hyperactivity disorder (ADHD) is a neurobehavioral disorder affecting children and adults. Cigarette smoking by pregnant women is associated with significant increase in ADHD risk for their offspring, suggesting a link between exposure of the developing brain to nicotine and ADHD risk. To model developmental nicotine exposure, we developed a mouse model of pre- and early postnatal (i.e. perinatal) nicotine exposure. Female C57/Bl6 mice received drinking water containing nicotine (100μg/ml)+saccharin (2%) or saccharin (2%) alone starting 3 weeks prior to breeding, throughout pregnancy and until 3 weeks postpartum (i.e. during the nursing period). Another group of female mice received plain drinking water without any additives. The offspring from each group were weaned 3 weeks after birth, and behavioral analyses were performed between 3 and 5 months of age. We examined spontaneous locomotor activity, working memory (using a Y-maze) and attention (using an Object Based Attention test; OBA). Perinatal nicotine exposure produced hyperactivity in female but not male mice, working memory and attention deficits in male but not female mice at 3 and 5 months of age. Exposure to saccharin alone did not produce significant changes in any of the measurements at either age. These data suggest that the perinatal nicotine exposure produces hyperactivity, working memory and attention deficit in a sex-specific manner. Our earlier work showed that nicotine exposure occurring during the 3 weeks prior to conception and lasting until birth (but not during the nursing period) produced hyperactivity, working memory deficit and attention deficit in both male and female mice. In this prenatal nicotine exposure model the offspring were cross fostered to drug-naïve nursing dams. In the present perinatal nicotine exposure mouse model the offspring remained with the nicotine+saccharin exposed or saccharin exposed biological mothers until weaning. Despite these differences in the design of the animal models, our data show that prenatal and perinatal nicotine exposures produce significant deficits in motor and cognitive phenotypes known to be associated with ADHD.

Disclosures: L. Zhang: None.

Poster

585. ADHD and Other Behavior Disorders

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Topic: A.07. Developmental Disorders

Support: FONDECYT 1140268

FONDECYT 11140535

Title: New insights into compensatory strategy of cortical processing in Attention Deficit Hyperactive Disorder

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Abstract: Attention Deficit Hyperactive Disorder (ADHD) is the most common neuropsychiatric disorder in infancy, affecting 5% to 10% of school-aged population. In order to investigate cerebral activation patterns involved in interference control in ADHD, a functional MRI study was conducted on 40 right-handed 10-12 year old boys, 20 ADHD combined type and 20 age and IQ-matched typical developing controls, while they were solving the multi source interference task (MSIT). The task presents a set of 3 digits to participants and they have to identify the number that is different from the other two. In the “congruent” condition (CC), the target number is placed congruently with its position on the response-pad, whereas in the “incongruent” condition (IC), the target number is never placed congruently with its position on the response-pad. Significant differences where observed in response accuracy between groups (Mixed ANOVA, Group factor: F=4.2, p=0.04) and interestingly, the MSIT effect measured as the reaction time (RT) between IC-CC contrast, was greater in CONTROL than in ADHD group (Control: 193.6ms, ADHD: 152.7ms, t31=2.3, p=0.02). The neuroimaging results for the MSIT effect showed a significant enhancement in the activation of cerebral areas involved in executive control for ADHD as compared to controls. Specifically, ADHD showed more activity in a cluster that includes both right Medial Frontal Gyrus and right Pars Opercularis of Inferior Frontal Gyrus (Mixed effect model, p-value=0.04, corr.). A psycho-physiological interaction (PPI) analysis was performed using this cluster as seed. By mean of this, we found that ADHD group presents a significant greater functional connectivity with bilateral orbitofrontal cortex (OFC) as compared to CONTROL group (Mixed effect model, p=0.02 corr.). A correlation analysis between performance in IC and connectivity between right Medial and Inferior Frontal Gyrus and bilateral OFC showed that a worse performance correlated positively with the

connectivity between these brain regions in both groups. Taken together our results could be interpreted in terms that the frontal activation elicited in MSIT by the ADHD represents a compensatory strategy resulting from the effort to maintain an adequate performance during the task. At the same time, our results can be interpreted in terms of underlying abnormalities in the switching between task positive and task negative networks.

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Poster

585. ADHD and Other Behavior Disorders

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Support: NeuroGenMRI (ERA-net PrioMedChild) grant to W. A.

Gambling, Dept. of Antidrug Policies c/o Presidency, Council of Ministries Italy, (grant to G. L. and W.A.)

Title: Enhanced limbic impaired cortical-loop connection onto hippocampus of NHE rats: application of resting state functional connectivity in a preclinical ADHD model

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Abstract: Due to a hyperfunctioning mesocorticolimbic system, the Naples-High-Excitability (NHE) rats have been proposed as a model for the mesocortical variant of ADHD. Compared to Naples Random-Bred (NRB) controls, NHE rats show hyperactivity, impaired non-selective attention(large number of rearing episodes with very low scanning duration), and impaired selective spatial attention (low performance in an eight-arm radial maze). Alteration in limbic functions has been proposed; however, resulting unbalance among forebrain areas has not been assessed yet. In the present work, by resting-state functional Magnetic Resonance Imaging (fMRI) in vivo, we investigated the connectivity of neuronal networks belonging to limbic vs.

cortical loops in NHE and NRB rats (n=10 each). Notably, resting-state fMRI was applied (3x10 min timeseries) using a multi-slice sagittal, gradient echo sequence. Voxel-wise connectivity maps at rest, based on temporal correlation among fMRI timeseries, were computed by seeding the hippocampus (Hip), nucleus accumbens (NAcc), dorsal striatum (dStr), amygdala (Amy) and prefrontal cortex (PFC) in both hemispheres. To summarize patterns of altered connections, clear directional connectivity was evident within the cortical loop, from PFC through the dStr and hence towards Hip: such communication was reduced.

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Poster

585. ADHD and Other Behavior Disorders

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CONICYT Fellowship to D.C./C.C.

Title: Impaired hippocampal plasticity and AMPA receptors phosphorylation in a mouse model of ADHD induced by prenatal nicotine exposure

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Abstract: Attention deficit hyperactivity disorder (ADHD) is the most common neurodevelopmental disorder characterized mainly by hyperactivity, impulsivity and inattention. Drugs used for the treatment of ADHD, such as Methylphenidate (MPH), improve cognitive behavior. We have shown that acutely applied MPH increases the TBS-dependent long tem potentiation (LTP) in CA3-CA1 synapses of hippocampus, promoting the insertion of AMPA receptors in the postsynaptic membrane of wild type rats. Little is known about the molecular

basis or modifications on LTP in mice models of ADHD. Using electrophysiological approaches and Western blot analysis we investigated LTP and AMPA receptor phosphorylation in an ADHD model induced by prenatal nicotine exposure (PNE). Female mice received nicotine 3 weeks before mating and during pregnancy (0.1 mg/ml nicotine + Saccharin 2%, in drinking water). Locomotor activity was assessed in PNE mice using an Open Field Test (OFT), working memory with Y-Maze and attention with Object-Based Attention test (OBA). LTP in CA3-CA1 was induced by applying theta burst stimulation (TBS, 5 trains, 100 Hz) in hippocampal slices obtained from Control and PNE mice (3-4 weeks old). Locomotor assessment show hyperactivity in PNE mice, total traveled distance in OFT was 81.8± 1.7m, n=8 compared with 55.4± 1.9m, n=8 in controls mice ($p<0.05$, Mann-Whitney U-test). Attention deficits are also evident in PNE mice; in OBA test these mice shown an impaired attention, since they spent more time with the known object (41.2 ± 1.3s, n=4) than the controls (4.3±0.3, n=4; $p<0.05$). As expected from hyperactive mice, Y-maze reveals higher number of entries in PNE mice (55.6±21, n=7) than controls(36.5±3.3, n=6; $p<0.05$), working memory assessments in this test shown reduced spontaneous arm entries (PNE: 32±3.1, n=7 ; Controls:47.2±3.7,n=6 ;$p<0.05$), increased alternate arm return (PNE: 33.3±2.3, n=7; Controls: 20.8±2.2,n=6 ;$p<0.05$) and decreased same arm returns(PNE: 14.6±1.2, n=7; Controls: 24.5±0.7,n=6 ;$p<0.05$). A reduced LTP was found in PNE mice (126±0.4%, n=8) compared with controls (151.2±0.5%, n=4,8;$p<0.05$). A significant decrease in phosphorylation ratio for GLUA1-Ser831 was found in PNE mice (0.67±0.05,n=5) versus controls mice (0.93±0.01,n=4;$p<0.01$). No significant change in the phosphorylation ratio of GLUA1-Ser845 was observed (PNE:0.55 ±0.9,n=5, control:0.6 ±0.12 n=4; $p > 0.05$).
Altogether our data indicate this model display all the behavioral hallmarks for ADHD: hyperactivity, impaired attention and memory deficits, which also are correlated with impaired LTP and lower phosphorylation of GLUA1-Ser831 subunit.

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Poster

585. ADHD and Other Behavior Disorders

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Topic: A.07. Developmental Disorders

Support: Jim and Betty Ann Rodgers Chair Fund

The Escher Fund for Autism

Title: Nicotine-induced epigenetic modification of the paternal germline DNA and ADHD symptoms in offspring

Authors: *D. M. MC CARTHY[1], S. E. LOWE[1], T. J. MORGAN, Jr[1], T. J. SPENCER[2], J. BIEDERMAN[2], P. G. BHIDE[1];
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Abstract: Cigarette smoking and other forms of tobacco use remain a leading cause of disease, disability and death in the United States. When a pregnant woman smokes cigarettes, her fetus is at risk for both physical and cognitive deficits. In fact, cigarette smoking during pregnancy nearly doubles the risk for ADHD in the offspring. While more men than women smoke cigarettes, little is known about the impact of father's nicotine use upon his offspring. With this in mind, we developed a paternal nicotine exposure mouse model in which adult male mice were exposed to nicotine (200μg/ml) in drinking water for 12 weeks. While the nicotine exposure was ongoing, the mice were bred with drug naïve females. To our surprise, the offspring of the nicotine-exposed fathers displayed hyperactivity and inattention, phenotypes commonly associated with ADHD. Interestingly, the nicotine-exposed mice (fathers) did not display either of these phenotypes. The offspring also displayed region- and sex-specific alterations in dopamine receptor mRNA expression in the brain. This led us to investigate the mechanisms underlying the expression of ADHD-related behavioral and molecular phenotypes in the offspring. One plausible mechanism is epigenetic modification of the father's germ cell DNA. We found a significant increase in DNA methylation in the nicotine-exposed fathers' spermatozoa. Specifically, DNA methylation was significantly altered in the dopamine D2 receptor promoter region. These data suggest that epigenetic modification of father's germline is associated with ADHD related behavioral and molecular phenotypes in the offspring.

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Poster

586. Adolescents: Human Imaging II

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Topic: A.09. Adolescent Development

Support: Pennsylvannia Department of Health

Title: A prospective EEG study of adolescent development demonstrates baseline disturbances in spatial working memory and associated cortical oscillations are predictive of substance use

Authors: *C. P. WALKER[1], N. POLIZZOTTO[2], N. RAMAKRISHNAN[2], Y. JHON[3], N. DAY[3], D. LEWIS[3], R. CHO[2];
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Abstract: Adolescence is a developmental period marked by substantial refinement of reward and impulse control circuitry in the brain. It has been hypothesized that increases in risk-seeking behavior seen in adolescents are a product of behavioral exploration and social and environmental learning that informs one's approach to social interaction. However, with increased risk-seeking comes the potential for negative impacts from behaviors such as illicit substance use. Currently, the manner in which early adolescent substance use affects brain maturation is unclear as much of our existing knowledge of the impact of adolescent substance use is based on retrospective studies. Therefore, we recruited a prospective cohort of 96 participants at 12 years of age who were determined to be high-risk for adolescent substance use, but had not reported any substance use at that time. Of this sample, 75 participants returned at 15 years, and 36 individuals were identified as having used either marijuana, alcohol, or tobacco. At the baseline and follow-up assessments, general demographic data and various cognitive batteries were collected. Participants also completed a spatial working memory task with a high vs. low load manipulation (3 vs 1 items) during an electroencephalographic (EEG) recording. Behavioral analyses identified a significant age by load by substance use interaction for error rates (ER), $p=.017$, which reflected a larger load effect at baseline for non-users compared to users. This effect remained after controlling for baseline differences in IQ, and the behavioral regulation index (BR), metacognition index (MI), and global executive composite (GEC) scores measured by the Behavior Rating Inventory of Executive Function (BRIEF). Additional main effects of age (ER) and load (ER & reaction time (RT)) were observed ($ps<.05$). A closer look at the BRIEF composite scores revealed significant age by substance use interactions for MI, $p=.007$, and GEC, $p=.024$ and a substance use main effect for BRI scores, $p=.003$, with substance users having higher scores overall or developing higher scores at later time points. Time-frequency analyses of the EEG data found group differences over central and parietal electrodes at 12 years with non-users showing a higher load-induced modulation of beta/gamma-band activity (20-40 Hz) during working memory maintenance, $p<.001$. This effect was absent in the substance use group, and these differences remained at the 15 year follow-up. Taken collectively, our data suggests that maturational differences in cortical high frequency oscillations may underlie a predisposition toward early adolescent substance use.

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Poster

586. Adolescents: Human Imaging II

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Topic: A.09. Adolescent Development

Support: CONACyT 619683/330142

Title: Local efficiency of brain's functional connectivity reflects longitudinal changes in temporal cortex lateralization in school-age children and adolescents

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Abstract: Lateralization is a dynamic process along age and unbalanced functional asymmetries are related with developmental disorders such as dyslexia. In order to improve the better understanding of normal and atypical development, this study focuses on characterize functional asymmetries in healthy school-age children and adolescents, and identify gender and age effects. The sample consists in two subsamples acquired during 2010 and 2015, respectively, all of them were healthy participants with resting state functional MRI scans (TR=2s; 150-300 volumes). First sample includes 66 subjects (6-10 years old, 36 girls), and second sample includes 44 subjects (11-19 years old, 34 girls), 32 of which participated in the first sample.
After standard preprocessing (no global signal regression), five noise-based components and movement-affected volumes (relative RMS > 0.25) were regressed out, and subjects with less than 120 non-affected volumes (4 minutes) were exclude from further analysis (6 subjects were excluded with this criteria).
For each subject, the average signal from 54 brain regions from each hemisphere was extracted and the Pearson correlation between all possible pairs of ipsilateral regions was obtained. A threshold of 0.35 was applied based on the best scale-free adjustment in a set of 0.05-step thresholds, and graph theory's Local Efficiency was obtained for each region in each hemisphere, then an asymmetry index was computed for each of the 54 unique bilateral regions. In order to consider the longitudinal aspects of the sample a linear mixed effects model was applied with age, gender, and their interaction as fixed effects and mean relative RMS as confounding variable. Significance effects were computed with a Likelihood Ratio Test and significance was defined as $p<0.05$ (corrected for multiple comparisons using false discovery rate at $q<0.05$). Significant effect was only found in the interaction of age and gender in the superior temporal gyrus (chisq=15.52; p=8.17e-05), where the rightward asymmetry increases with age only in males. No gender or age effects alone were found.

This study showed functional connectivity asymmetries in a region surrounding the Sylvian fissure, which is related with auditory and speech processing. In our results this effect was only evident along the interaction with the age, so this asymmetry could be established during the adolescent period when language differences between genders are more patent. The hemispheric asymmetries in functional connectivity described in this study contribute to the better understanding of normal development.
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Poster

586. Adolescents: Human Imaging II

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Topic: A.09. Adolescent Development

Title: Differences in neural substrates of aversive face processing in pre-pubertal and pubertal girls and boys

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Abstract: Adolescence is a time of significant change in social behavior and emotional regulation. During this time, individual and sex-specific differences are seen in developmental trajectories of brain maturation. One hypothesis is that the process of puberty, including increases of gonadal and adrenal hormones, may be linked to both structural and functional brain maturation. Of particular interest is the sex difference in the developmental trajectory of emotion processing. Previous research has shown both structural and functional sex differences in brain regions linked to socioemotional processing. Here, we examined sex and pubertal stage effects on neural processing while viewing emotional faces in well-characterized, healthy children. Fifty four typically developing children participated in the study (26 girls), all having a body

mass index between the 15th and 85th percentiles, and skeletal age within two standard deviations of age-specific population norms as determined by wrist x-rays. Pubertal development was assessed by physical examination of breast (girls) and testicular development (boys) and participants were categorized into two groups: pre-pubertal (N=26, 8.2 ± 0.1 years, 13 girls) and pubertal (N=28, 12.6 ± 0.1 years, 13 girls). Functional MRI data were collected on a 3T GE scanner while participants viewed blocks of aversive and non-aversive faces. Data were pre-processed in SPM5, normalized to a study-specific MNI template using ANTS, spatially smoothed (8 mm), and motion corrected using ART. Whole-brain voxel-wise analyses in AFNI used a multivariate model to determine the main and interaction effects of sex and pubertal status on BOLD signal during viewing of aversive versus non-aversive faces.
Across both groups, there was a main effect of sex during aversive face viewing such that girls showed greater activation than boys in the right intraparietal sulcus, left orbitofrontal cortex (OFC), left dorsolateral prefrontal cortex (DLPFC), right superior temporal sulcus, left anterior cingulate cortex (ACC), and right posterior cingulate cortex ($p<0.001$ FDR). There were no regions in which boys showed greater activation than girls, no main effects of pubertal status, and no interactions between sex and pubertal status. These data are consistent with previous findings in adults showing greater activation in women than men in the ACC, OFC, and DLPFC. These results also suggest that some sex differences in neural processing related to social and emotional behaviors may be established prior to puberty and may develop independently of gonadal sex-steroid secretion.

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Poster

586. Adolescents: Human Imaging II

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Topic: A.09. Adolescent Development

Title: Modulation of response inhibition by puberty and sex: a cross-sectional examination of neural recruitment during a stop-signal task in children

Authors: *M. ZAWADZKI[1], K. M. REDING[1,2], S. WEI[1], T. A. NASH[1], Y. TONG[1], S. MURRAY[1], T. NGUYEN[1], H. A. RAAB[1], P. E. MARTINEZ[2], D. E. BOYLE[3], J. S.

KIPPENHAN[1], P. D. KOHN[1], S. J. SOLDIN[4], L. K. NIEMAN[5], J. A. YANOVSKI[6], P. J. SCHMIDT[2], K. F. BERMAN[1];
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Abstract: The capacity to inhibit a pre-potent response is a crucial executive function that matures over the course of development. Poor impulse control during adolescence has been explained by a dual-systems model in which rapid development of limbic regions precedes that of cognitive control areas. The Stop-Signal task, which requires the participant to withhold an already-initiated response, is a reliable metric of inhibition. The current fMRI study investigated the effects of pubertal development and sex on neural recruitment during response-inhibition in healthy children and adolescents.

Fifty-two typically-developing children and adolescents performed the Stop-Signal task in a 3T GE MRI scanner. Participants were divided into two pubertal groups based on clinician-determined Tanner Stage: 27 Tanner Stage 1 pre-pubertal children (8.7$\pm$0.3 yrs, range=8.1-9.3; 12 girls) and 25 Tanner Stage 2-5 adolescents (13.1$\pm$0.7 yrs, range=12.2-14.6; 9 girls). Data were pre-processed and registered to a study-specific template in MNI space using SPM, and motion-corrected using ART. Using AFNI, we compared whole brain voxel-wise BOLD signal during inhibition (i.e. Successful-Stop events) to that of non-inhibition trials (i.e. Go events) to test for main and interaction effects of sex and pubertal group.

Pre-pubertal children had slower average reaction times to Go signals than pubertal children (633$\pm$76 ms vs 583$\pm$82 ms, p=0.014), as well as fewer completed Go trials (83.8$\pm$.12% vs 90.9$\pm$0.05%, p=0.012). Girls had slower go reaction times than boys (636$\pm$76 ms vs 590$\pm$69 ms, p=0.028) and higher stop accuracy (52$\pm$0.13% vs 42$\pm$0.15%, p=0.015). In fMRI, there was more robust BOLD signal in Successful-Stop than in Go trials in bilateral inferior frontal gyrus, supplementary motor area, and subthalamic nucleus, as well in visual processing areas ($p<1x10^{-7}$, FDR). Main effects of pubertal group and sex were not significant, but a pubertal group by sex interaction was found in in the right medial prefrontal cortex ($p<0.06$, FDR). Post-hoc analyses showed that in prepubertal girls this area is deactivated, but becomes activated following the onset of puberty ($p<0.001$). This pattern was opposite in boys (p=0.03).

Neither pubertal stage nor sex significantly affected activation within regions typically involved in response inhibition. However, a sex-by-pubertal stage interaction was observed in a region important in social/emotional function. The fact that sex differences in mPFC activity were observed before puberty and were altered with puberty suggests an early dimorphism that is independent of gonadal steroids, but responsive to age or to the endocrine events of puberty.

Disclosures: **M. Zawadzki:** None. **K.M. Reding:** None. **S. Wei:** None. **T.A. Nash:** None. **Y. Tong:** None. **S. Murray:** None. **T. Nguyen:** None. **H.A. Raab:** None. **P.E. Martinez:** None. **D.E. Boyle:** None. **J.S. Kippenhan:** None. **P.D. Kohn:** None. **S.J. Soldin:** None. **L.K. Nieman:** None. **J.A. Yanovski:** None. **P.J. Schmidt:** None. **K.F. Berman:** None.

Poster

586. Adolescents: Human Imaging II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 586.05/E17

Topic: A.09. Adolescent Development

Support: NIH/NIDA T32 5T32DA024635-07

NSF 1606979

Title: Motivation, goals, and intelligence mindset in the adolescent striatum during feedback-based learning

Authors: *S. DEPASQUE, A. GALVAN;
UCLA, Los Angeles, CA

Abstract: Performance-related feedback is an important tool used by educators to help adolescents learn. However, the learning efficacy of such feedback depends upon motivational factors that can vary within individuals and across situations. Corticostriatal brain systems play a critical role in both motivation and learning, so it is important to understand how well-established ontogenetic changes in these systems during adolescence might scaffold learning and influence susceptibility to motivational factors. The present study used fMRI in conjunction with a motivational manipulation to compare adolescents' (n=15, M_{age}=13.5) and adults' (n=13, M_{age}=25.3) neural and behavioral responses to performance-related feedback. Participants completed a learning task in three phases: 1) a study phase outside of the scanner, 2) a scanned feedback phase, 3) and a post-test immediately after the scan. The feedback phase was divided into blocks of trials, half of which were framed as a test (threat) and half as opportunities to practice learning strategies (nonthreat). In both conditions, adults outperformed the adolescents, across both the feedback and post-test phases. However, on the post-test, adolescents performed as well as adults on items that were previously answered incorrectly, suggesting that they were just as capable of correcting their errors based on feedback. For items learned under the threat condition, adolescents exhibited even higher numbers of corrected errors than adults. On average, the motivational condition did not influence task performance for either age group; however, in youth, normative goals (i.e., goals to outperform others) were associated with greater effects of the threat manipulation on the correction of errors. In other words, teens who aimed to outperform their peers showed a greater threat-related improvement in the correction of incorrect responses. This relationship was not observed in adults. Feedback engaged similar brain networks in both age groups, including the striatum, medial prefrontal cortex, and posterior cingulate. ROI analyses identified age by threat interactions in the nucleus accumbens, caudate nucleus, amygdala, and hippocampus. In both adults and adolescents, neural responses to the threat manipulation related to participants' beliefs about intelligence: individuals who believe

intelligence is fixed exhibited stronger effects of threat in the striatum. These results highlight the importance of considering the interaction between contextual factors and individual differences when studying developmental influences on learning.

Disclosures: **S. Depasque:** None. **A. Galvan:** None.

Poster

586. Adolescents: Human Imaging II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 586.06/E18

Topic: A.09. Adolescent Development

Support: NIH Grant P41EB015922

NIH Grant U54EB020406

Title: Effects of Val158Met catechol-O-methyltransferase polymorphism on structural striatocortical connectomes in children and adolescents: a neuroimaging PheWAS

Authors: ***L. ZHAO**, K. CLARK, C. GONZALEZ-ZACARIAS, F. SEPEHRBAND, A. TOGA;
USC, Los Angeles, CA

Abstract: Introduction: The Val158Met (or known as rs4680) polymorphism within the catechol-O-methyltransferase (COMT) gene results in valine (Val) to methionine (Met) substitution, with different dopamine release in subcortical regions, e.g. the striatum, and the cortex in Val158 and Met158 carriers. The variations in dopamine levels influenced by Val158Met has been linked to changes in cognitive functioning. Few studies have examined possible neuroanatomic effects. This work investigated effects of Val158Met on structural brain connectomes using a phenome-wide association study (PheWAS) approach, i.e. to identifiy which brain phenotypes are influenced by a genotype of interest.
Methods: We obtained volumetric measures of subcortical regions and surface-based morphometric (SBM) measures of the cortex (including the cortical thickness, surface area, volume, gyrification index and gray to white matter ratio) from structural magnetic resonance imaging (MRI) scans of two neurodevelopmental cohorts: 1. the Pediatric Imaging, Neurocognition, and Genetics (PING) dataset (464 subjects, age=11.69±4.84, 223 females); 2. the Philadelphia Neurodevelopmental Cohort (PNC) (662 subjects, age=14.79±3.35, 331 females). Structural striatocortical networks were constructed by respectively correlating the caudate and putamen volumes with the SBM measures across the cortex. Effects of Val158Met

on the structural connectomes were assessed with analysis of variance (ANOVA).
Results: As expected, Val158Met explained a significant amount of variance in the striatal connectivities with the lateral prefrontal cortex (PFC), which was found in the putamen-to-cortical thickness network in the PNG cohort and in the caudate-to-cortical volume network in the PNC cohort. This may account for the changes in cognitive performance related to dopamine level variations in different Val158Met carriers. Additionally, in PFC cohort, significant genetic effects on the caudate-to-cortical volume correlations were also found in the insula, the cingulated, temporal and visual cortices. This is in line with previous findings that Val158Met is associated with processing of sensory emotion and pain and with the brain development in these areas.
Conclusion: This work yields new insight into the mechanisms of how the COMT gene influences the brain in children and adolescents, and especially illustrates that Val158Met plays an important role in determining the biological factors that underlie structural striatocortical connectomes.

Disclosures: **L. Zhao:** None. **K. Clark:** None. **C. Gonzalez-Zacarias:** None. **F. Sepehrband:** None. **A. Toga:** None.

Poster

586. Adolescents: Human Imaging II

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Program#/Poster#: 586.07/E19

Topic: A.09. Adolescent Development

Support: NIAAA Grant 5R01AA018405 (D.S.O.)

Title: Reduced frontal and parietal cortical thickness in adolescents with a family history of alcohol use disorder

Authors: *K. E. RASMUSSEN[1], J. G. VAIDYA[2], J. KRAMER[2], S. KUPERMAN[2], D. LANGBEHN[2], D. S. O'LEARY[2];
[1]Interdisciplinary Grad. Program in Neurosci., [2]Psychiatry, The Univ. of Iowa, Iowa City, IA

Abstract: Individuals with a family history (FH+) of alcohol use disorder (AUD) are 2 to 5 times more likely to abuse alcohol in their lifetimes than individuals with no family history of AUD (FH-) (Spadoni et al., 2008). Even in the absence of heavy alcohol use, FH+ adolescents tend to perform worse than their FH- peers on cognitive, emotional, and decision-making tasks, perhaps due in part to underlying neurobiological differences (Acheson et al., 2009). The purpose of this study is to determine whether FH+ individuals show neuroanatomical differences

compared to FH- controls. Here, individuals with at least one biological parent diagnosed with AUD are defined as FH+, and those with no biological parent with AUD are defined as FH-. By design, our sample had limited or no past substance use thereby minimizing confounds due to alcohol consumption. Structural brain scans of 94 FH- and 95 FH+ adolescents aged 13-18 were obtained using magnetic resonance imaging (MRI). Measurements of cortical thickness were obtained using the FreeSurfer software package. Subjects were binned into one of three age categories for analysis (13-14, 15-16, or 17-18 years). Analysis of covariance (ANCOVA) was conducted for each region of interest using SPSS software with age bin and FH group as independent variables and gender and total intracranial volume (ICV) as covariates. First, individual cortical lobes (frontal, parietal, occipital, and temporal) were analyzed. Then, any lobes showing group effects were broken down into individual regions for further analysis. Lobe level analysis showed group effects ($p<0.10$) for the frontal and parietal lobes. Follow-up analyses within these lobes demonstrated that FH+ had, on average, reduced cortical thickness in the in the right lateral and medial orbitofrontal cortices of the frontal lobe ($p<0.05$) as well as in the right postcentral region of the parietal lobe ($p<0.10$). These main effects were qualified by a significant interaction with age ($p<0.05$). FH+ subjects had thinner cortices across the age range, however the declines in thickness were more dramatic in FH- subjects. These findings demonstrate that the frontal and parietal lobes of adolescents are affected by family history of AUD. Decreased cortical thickness in FH+ at early adolescence may indicate deficient development prior to adolescence, and reduced cortical thinning during adolescence may reflect lack of pruning in these regions. As the frontal and parietal lobes are associated with executive functions and behavioral regulation, improper development of these regions may lead to a greater susceptibility to substance use disorders.

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Poster

586. Adolescents: Human Imaging II

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Topic: A.09. Adolescent Development

Support: Wellcome Trust (NeuroScience in Psychiatry Network)

NIH-OxCam Scholars Program

Title: Morphometric similarity networks: a novel integrative method for studying inter-individual differences in brain structure

Authors: *J. SEIDLITZ[1,3], F. VÁŠA[1], M. SHINN[1], R. ROMERO-GARCIA[1], K. WHITAKER[1], P. VÉRTES[1], F. LALONDE[3], A. RAZNAHAN[3], E. BULLMORE[1,2,4,5]; [1]Brain Mapping Unit, [2]Behavioural and Clin. Neurosci. Inst., Univ. of Cambridge, Cambridge, United Kingdom; [3]Developmental Neurogenomics Unit, Natl. Institue of Mental Hlth., Bethesda, MD; [4]Cambridgeshire and Peterborough NHS Fndn. Trust, Huntingdon, United Kingdom; [5]Academic Discovery Performance Unit, GlaxoSmithKline, Stevenage, United Kingdom

Abstract: There is a large body of work demonstrating profound inter-individual differences in cortical brain structure, as measured by magnetic resonance imaging. Recent research suggests that these inter-individual differences in the structure of a given brain region co-vary with inter-individual differences in the structure of other brain regions, referred to as structural covariance (SC). However, compared to studies of functional brain networks, where a brain network is derived for each subject in a cohort, SC has been limited to analyses at the group-level due to the use of a single morphometric feature (e.g. cortical thickness) in the generation of a brain network. Although some methods have been proposed to generate individual SC networks, these have still been restricted to the use of a single morphometric feature, and have not made use of other aspects of brain morphometry, such as regional brain curvature and cortical white matter content. Thus, using a multiparametric MRI sequence in a cohort of 296 typically developing adolescents (148 females), we extracted 10 morphometric features for each brain region in a 308-region parcellation, capturing diverse aspects of cortical morphometry. We constructed connectivity matrices using the pairwise Pearson correlation of the scaled regional morphometric features, resulting in individual 308x308 morphometric similarity networks (MSN). Network analyses of the individual MSNs produced graph metrics similar to those of traditional structural and functional brain networks (i.e. small-world and highly modular), and yielded a consensus modular decomposition which recapitulated the lobar organization of the brain. Edgewise analysis at the group level revealed differences in the spatial patterning of the most extreme left-tail (negative) and right-tail (positive) edges, with the former mediating primarily inter-modular and long distance connections, and the latter mediating intra-modular and inter-hemispheric connections (bilaterally homologous regions). A significant decrease in individual global brain similarity - the absolute edgewise mean of each MSN - was observed with increasing age, suggesting that brain regions are becoming morphometrically differentiated during adolescence. Overall, by providing a new approach to measure regional morphometric relationships across the whole brain in a single subject, we believe this method will help us better understand the normative changes that occur during neurodevelopment, and will be sensitive to the individual differences in brain structural network organization that are a result of disease-specific processes or psychopathological abnormalities.

Disclosures: J. Seidlitz: None. **F. Váša:** None. **M. Shinn:** None. **R. Romero-Garcia:** None. **K. Whitaker:** None. **P. Vértes:** None. **F. Lalonde:** None. **A. Raznahan:** None. **E. Bullmore:** A. Employment/Salary (full or part-time): GlaxoSmithKline (part-time).

Poster

586. Adolescents: Human Imaging II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 586.09/E21

Topic: A.09. Adolescent Development

Support: MH067924

Title: The short timescale neural contributions to resting state correlations

Authors: *D. F. MONTEZ[1], S. MAREK[1], B. LUNA[2];
[1]Neurosci., [2]Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Study: fMRI studies have revealed the underlying network structure of the resting brain and have informed our understanding of how networks continue to develop during adolescence. Very little is known about the time scales of neuronal activity that underlay the BOLD signal correlations typically analyzed in fMRI resting state analyses. Here, we leverage combined measurements of fast time scale MEG correlations and fMRI correlations within the same set of subjects to understand the temporal structure of neural activity observed indirectly in BOLD signal correlations. Methods: *MEG:* We collected 5 minutes of eyes open fixation resting state MEG (1000Hz sampling frequency) and fMRI (1Hz sampling frequency) data from 33 subjects, ranging in age from 14-31 years. We extracted BOLD and MEG time series from a set of 333 cortical ROIs from each of the subjects. To characterize the temporal structure of MEG correlations, we computed cross-correlograms for each ROI pair across a 10 second interval. Next we computed the relationship between each lag of the MEG cross-correlograms to their corresponding BOLD signal correlations. This analysis reveals the temporal structure of MEG correlations that contribute to BOLD signal correlations. Results**:** We find that BOLD signal correlations across the cortex reflect structured temporal correlations occurring on time scales that are much faster that the typical BOLD signal sampling rates. In particular, higher BOLD signal correlations are associated with rhythmic 1Hz and 10Hz oscillations, as well as relatively short time scale synchronous correlations.

Disclosures: **D.F. Montez:** None. **S. Marek:** None. **B. Luna:** None.

Poster

586. Adolescents: Human Imaging II

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Program#/Poster#: 586.10/E22

Topic: A.09. Adolescent Development

Support: NSF BCS0963750

Title: Sleep loss amplifies the relationship between stress and neural correlates of risky decision-making in adolescents and adults

Authors: *J. PHUONG, A. GALVAN;
Psychology, UCLA, Los Angeles, CA

Abstract: Insufficient sleep has been associated with increased risk-taking and poor decision-making. Sleep deprivation has been shown to amplify physiological responses to stress, but it is yet unknown how sleep deprivation interacts with acute stress to affect the neural correlates of risky decision-making in adolescents. In the current study, daily self-reports of stress were documented in adolescents (n = 22; ages 15-17 years) and adults (n = 22 adults; ages 25-30 years). Participants also self-reported their recent sleep duration and completed two fMRI visits during which they performed an adaptive risky decision-making task: once each when they endorsed a high and low level of stress. Results revealed that under high stress, decreasing hours of sleep per night was associated with more disadvantageous risks and more gain-focused risks in both adolescents and adults. In addition, as sleep duration decreases, ventromedial prefrontal cortex activation increases when deciding not to take disadvantageous risks; this effect was stronger in adolescents than in adults. Sleep duration was not associated with risky decision-making or neural activation under low stress, suggesting that insufficient sleep may amplify the effects of stress and affect decision-making behavior through modifications of the prefrontal cortex, a region undergoing significant development during adolescence.

Disclosures: J. Phuong: None. **A. Galvan:** None.

Poster

586. Adolescents: Human Imaging II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 586.11/E23

Topic: A.09. Adolescent Development

Title: Receptive verbal ability in autism spectrum disorder: a preliminary fmri investigation

Authors: *S. HUEMER[1], F. KRUGGEL[2], V. MANN[3], J.-G. GEHRICKE[4];
[1]Psychology, Loyola Marymount Univ., Los Angeles, CA; [2]Med. Bioengineering, [3]Cognitive Sci., [4]Pediatrics, Univ. of California Irvine, Irvine, CA

Abstract: Introduction: The present fMRI experiment compares brain activity of adolescents with Autism Spectrum Disorders (ASD) to those of typically developing adolescents as they listen to their own first names, familiar people's names, numbers, and names objects of high interest. Carmodey et al. (2007) investigated hearing one's name in ASD in an fMRI study with a 4-year-old sedated girl with ASD and found that the greatest volume of overall activation was recorded in response to numbers as compared to her first name and the word 'hello'. **Methods:** 9 participants with ASD and 9 controls (12 - 20 yrs) were enrolled, the diagnosis confirmed, and the Peabody Picture Vocabulary Test (PPVT-III), a test for verbal ability, administered. Subjects passively listen the stimuli. T1-weighted MR images were acquired on a Philips Achieva 3T scanner. T2-weighted images were acquired using a single-shot EPI protocol. During each of the three functional imaging sequences, 110 volumes were taken. Spm software was used for data anlysis, $p < 0.05$ and $p < 0.1$. **Results:** When hearing their own name, the ASD group showed overall less areas of activation and they relied more heavily on prefrontal structures (especially the frontal pole, BA 10) as compared to controls in support of the findings from Carmody et al., 2007. Interestingly, we also found activity in the left thalamus in the group that scored lower on the verbal ability measure, the PPVT.
When hearing their own name, controls and the individuals who scored higher on the verbal ability measure, the PPVT, (HS group) showed activation in areas of self-referential processing and areas associated with hearing one's name (including BA 7, BA 9, BA 13, BA 17, BA 22, BA 31, BA 19). Also notable was a reliance on more anterior regions in the ASD and the lower-scoring (LS group) vs. the more posterior activation in the control and HS groups. Interestingly, we found an involvement of the right hippocampus (BA 53) in the HS group. **Conclusions:** Overall, our results showed that, when listening to our highly self-referential stimuli, the neurotypical adolescents relied more on brain regions associated with self-recognition that were expected to be activated when hearing their own name and stimuli of high self-referential value. Adolescents with an ASD, on the other hand, relied on frontal-occipital areas linked to visual memory recognition and episodic memory.
We infer that adolescents with ASD, especially those with lower verbal abilities, may

"remember" their own name like they remember another familiar person's name, a desirable familiar object, or a number but they may not implicitly "know" self-referent words as distinct from other words.

Disclosures: **S. Huemer:** None. **F. Kruggel:** None. **V. Mann:** None. **J. Gehricke:** None.

Poster

586. Adolescents: Human Imaging II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 586.12/E24

Topic: A.09. Adolescent Development

Support: Fellowship CONACYT for Masters degree.

Title: Functional connectivity changes throughout childhood to adolescence

Authors: *E. NAVARRETE, Z. GRACIA, F. BARRIOS, S. ALCAUTER;
Lab. C-12, Inst. De Neurobiología., Querétaro, Mexico

Abstract: Introduction: Adolescence is an important transition in the life of human beings, characterized by rapid growth and pace changes, involving biological, cognitive and psychosocial aspects. The study of brain functional development during this period contributes to the better understanding of the brain-behavior relation. Here, we explore the developmental properties of the brain network based on measures of functional connectivity (resting state fMRI) and methods from graph theory.
Methods: The sample consisted in two subsamples acquired during 2010 and 2015, respectively, all of them were healthy participants with resting state functional MRI scans (TR=2s; 150-300 volumes). First sample included 66 subjects (6-10 years old, 36 girls), and second sample included 44 subjects (11-19 years old, 34 girls), 32 of which participated in the first stage. After standard preprocessing (no global signal regression), five noise-based components and movement- affected volumes (relative RMS > 0.25) were regressed out, and subjects with less than 120 non-affected volumes (4 minutes) were exclude from further analysis (6 subjects excluded). For each subject, the average signal from 116 brain regions (AAL atlas)was extracted and the Pearson correlation between all possible pairs of regions was obtained. A threshold of 0.35 was applied based on the best scale-free adjustment in a set of 0.05-step thresholds, and graph theory's weighted degree was obtained for each region. A linear mixed effects model was fitted, with age, gender, and their interaction as fixed effects and mean relative RMS as confounding variable. Significance effects were defined as $p<0.05$ (corrected for multiple comparisons using false discovery rate at $q<0.05$).

Results: We observed a significant negative effect of WD with age (i.e., more age less WD) in 56 regions, mainly including the frontal and parietal lobes and cerebellum. Of these regions, 14 were frontal and 5 parietal.
Conclusions: The decrements of WD with age, suggest functional specialization, which may be related to the synaptic pruning during adolescence. It is possible that the decrease of the WD on parietal and frontal regions, obey the process of massive elimination of short-range connections that occurs concomitantly with selective strengthening of long-range connections, which would result in a more efficient network and give raise to the complex reasoning seen in this period.

Disclosures: E. Navarrete: None. **Z. Gracia:** None. **F. Barrios:** None. **S. Alcauter:** None.

Poster

586. Adolescents: Human Imaging II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 586.13/E25

Topic: A.09. Adolescent Development

Title: Examining reward and cognitive control resting state connectivity using extended unified structural equation modeling across development

Authors: *N. ROBERTS[1], L. LO[1], R. WHITE[1], B. LUNA[2], C. F. GEIER[1];
[1]Human Develop. and Family Studies, The Pennsylvania State Univ., State College, PA; [2]The Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Characterizing the interactions between reward and inhibitory control brain systems are critical to gain a fuller understanding of decision-making across the lifespan. Individual differences in resting state functional connectivity (rs-fcMRI) patterns can predict performance on a range of cognitive tasks, as well as individual differences in BOLD activity during tasks. However, relationships between rs-fcMRI and incentivized IC task performance have yet to be fully characterized. The current study applies extended unified structural equation modeling (euSEM) to rs-fcMRI data in children, adolescents, and adults in order to investigate developmental and individual differences in these patterns. In addition, we examined whether these differences are predictive of performance on an incentivized oculomotor anti-saccade task. Typically developing individuals (N=43, ages 10-18) completed a resting state scan and an incentivized oculomotor anti-saccade task. Exploratory euSEM provides confirmatory tests of a priori factor structures, relationships between latent factors and multigroup tests of mean structure measurement invariance. This approach allows for the recovery of effective connectivity maps for both groups and individuals and estimates lagged and contemporaneous directed paths between brain regions of interest (ROIs) simultaneously. ROIs were selected

based on previous literature delineating regions involved in incentive processing (e.g. NAcc, amygdala), saccade generation (FEF, PPC), and inhibitory control (infPCS, vlPFC). Our preliminary results indicate higher contemporaneous resting state connectivity (RSC) between right and left (right -> left) vlPFC, and that this is associated with slower reaction times (RTs) on reward, loss and neutral trials. Children, relative to adolescents, have significantly slower RTs in all three conditions (no significant difference between adolescents and adults). Greater lagged connectivity in fefl and ppcr was associated with slower RTs on reward and loss trials in children relative to adolescents. Adults demonstrated greater contemporaneous connectivity between PPC left -> PPC right, and this was associated with faster reaction times on loss trials. Finally, compared to adults, children had greater resting activity from ppcr -> ppcl. Our results highlight specific differences in connectivity and their relationship to behavior across development.

Disclosures: **N. Roberts:** None. **L. Lo:** None. **R. White:** None. **B. Luna:** None. **C.F. Geier:** None.

Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 587.01/E26

Topic: B.03. G-Protein Coupled Receptors

Support: BBSRC Grant BB/H530570/1

Title: Functional diversity of group II (mGlu2 and mGlu3) metabotropic glutamate receptors in the thalamus.

Authors: *C. S. COPELAND[1], T. M. WALL[2], S. A. NEALE[3], E. NISENBAUM[2], T. E. SALT[1];
[1]Univ. Col. London, London, United Kingdom; [2]Lilly & Co, Indianapolis, IN; [3]Neurexpert Ltd., London, United Kingdom

Abstract: The ventrobasal thalamus (VB) and thalamic reticular nucleus (TRN) transmit somatosensory inputs to S1 cortex. This circuitry can mediate sensory discrimination, enabling relevant information to be discerned from background activity. Group II metabotropic glutamate (mGlu) receptors (subtypes mGlu2/mGlu3) located within this circuitry can modulate somatosensory transmission. However, due to a lack of subtype selective compounds, the relative contributions of the two subtypes to this overall Group II receptor effect remains unclear. We address this using electrophysiology techniques and novel pharmacological tools. Whole-cell recordings were made *in vitro* from VB relay neurons in brain slices from Sprague-

Dawley rats, wild-type and mGlu3 knock-out mice. The Group II agonist LY354740 presynaptically suppressed GABAergic IPSCs arising from TRN in both rat and wild-type mice slices, but had no effect upon IPSCs recorded from mGlu3 knock-out mice. This suggests that the presynaptic modulation of inhibition is predominantly mGlu3 receptor mediated. We then made extracellular single-neuron recordings *in vivo* with multi-barreled iontophoretic electrodes in the VB of anaesthetized Wistar rats. Single vibrissae deflections to activate single VB neurons were performed and compounds locally applied. The action of the dual mGlu2 agonist/mGlu3 antagonist LY395756 provided further evidence that the Group II effect on somatosensory transmission is largely mediated via mGlu3: when applied alone the predominant LY395756 effect mimics the Group II antagonist LY341495 under the same conditions. However, when LY395756 was co-applied with the mGlu2 selective positive allosteric modulator (PAM) LY487379, an increase in responses to vibrissa deflection was observed, an effect similar to the Group II agonist LY354740 under the same conditions. This can be attributed to the PAM revealing the underlying LY395756 mGlu2 agonism component.
These results show that mGlu2 and mGlu3 receptors have different actions in the VB thalamic circuit, with mGlu3 directly modulating GABA release from TRN terminals. This heterogeneity in Group II receptor physiology bears consequence, as compounds active exclusively at mGlu2 are unlikely to perturb maladapted VB firing patterns associated with somatosensory aberrations, such as those described in psychiatric disease. Targeting activity at mGlu3 receptors is likely more appropriate. Indeed, polymorphisms in mGlu3, but not mGlu2, gene alleles have been detected in patients with schizophrenia.

Disclosures: C.S. Copeland: None. **T.M. Wall:** None. **S.A. Neale:** None. **E. Nisenbaum:** None. **T.E. Salt:** None.

Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 587.02/E27

Topic: B.03. G-Protein Coupled Receptors

Title: The kynurenine metabolite, xanthurenic acid, a putative regulator of glutamatergic neurotransmission, regulates vascular tone

Authors: *F. FAZIO[1], A. CARRIZZO[1], L. LIONETTO[2], A. D'AMATO[1], M. SIMMACO[2,3], M. CANNELLA[1], G. BATTAGLIA[1], F. NICOLETTI[1,4], C. VECCHIONE[1,5];
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Abstract: The kynurenine pathway (KP) of tryptophan metabolism generates a plethora of compounds acting on different targets. Xanthurenic acid (XA), a kynurenine metabolite that has long been considered as part of the detoxification process of 3-hydroxykynurenine (3-HK), has recently emerged as a putative neurotransmitter in the central nervous system. While the mechanism of action of XA is not entirely clarified, some of the effects mediated by XA involve type-2 metabotropic glutamate receptors (mGlu2 receptors). Recently, several kynurenine derivatives have been tested as hypotensive agents, and L-kynurenine itself has been identified as an endothelium-derived factor that strongly influences the regulation of vascular tone during inflammation (Wang et al., Nat. Med. 16:279-285, 2010). Here, we examined the vasoactive properties of XA for the potential role of this compound in mechanisms of neuroinflemmation and associated vascular changes. We found that: i) XA (2.5 to 1000 μmol/l) almost completely abolished phenylephrine-induced constriction of mesenteric arteries both in the presence or in the absence of the endothelial layer; ii) under the same conditions, L-kynurenine behaved similarly to XA but with lower efficacy and potency; iii) XA was unable not able to relax aorta preconstricted with phenylephrine; iv) in conscious C57BL/6N mice (n = 5) XA (10 and 100 mg/kg) reduced blood pressure; v) in mice pulsed with lipopolysaccharide (LPS) (10 mg/kg, i.p.), levels of kynurenines and XA (detected by HPLC/mass-mass spectrometry) raised by about tenfold at 36 hours; vi) mice pretreated with RO 61-8048 (an inhibitor of kynurenine monooxygenase that transforms L-kynurenine in the XA precursor, 3-HK) did not respond to LPS with a reduction of blood pressure. These data suggest that XA is involved in the regulation of the vascular tone in mice.

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Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 587.03/E28

Topic: B.03. G-Protein Coupled Receptors

Title: Release regulating mGlu2-preferring and mGlu3 preferring autoreceptors in cns

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Abstract: Presynaptic, release-regulating metabotropic glutamate 2 and 3 (mGlu2/3) autoreceptors exist in central nervous system (CNS). They represent suitable targets for therapeutic approaches to central diseases that are typified by hyperglutamatergicity. The availability of specific ligands able to differentiate between mGlu2 and mGlu3 subunits allows to further characterize these autoreceptors. This study aims at investigating the pharmacological profile of mGlu2/3 receptors in selected CNS regions and at evaluating their functions in mice suffering from experimental autoimmune encephalomyelitis (EAE).
The comparative analysis of presynaptic mGlu2/3 autoreceptors was performed by analyzing the effect of selective mGlu2/3 receptor agonist(s) and antagonist(s) on the release of [^{3}H]-D-aspartate from cortical and spinal cord synaptosomes in superfusion. Experiments were also carried out to analyze mGlu2/3 autoreceptor-mediated releasing functions in EAE animals and whether *in vivo* LY379268 administration can restore impaired glutamate release in these mice. Western blot analysis and confocal microscopy confirmed the presence of presynaptic mGlu2/3 receptor proteins. Cortical synaptosomes possess LY541850-sensitive, NAAG-insensitive autoreceptors having low affinity for LY379268, while LY541850-insensitive, NAAG-sensitive autoreceptors with high affinity for LY379268 exist in spinal cord terminals. In EAE mice, mGlu2/3 autoreceptors lost completely their inhibitory activity in cortical, but not in spinal cord synaptosomes. *In vivo* LY379268 (1-0.01 mg kg^{-1}) administration restored glutamate exocytosis capability in spinal cord but not in cortical terminals. We propose the existence of mGlu2-preferring and mGlu3-preferring autoreceptors in mouse cortex and spinal cord, respectively. The mGlu3-preferring autoreceptors could represent a target for new pharmacological approach for demyelinating diseases.

Disclosures: **A. Pittaluga:** None. **T. Bonfiglio:** None. **G. Olivero:** None. **C. Cervetto:** None. **M. Grilli:** None. **C. Usai:** None. **M. Marchi:** None.

Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 587.04/E29

Topic: B.03. G-Protein Coupled Receptors

Support: JTC2012 LIGHTPAIN

Title: Unraveling a role for thalamic mGlu5 receptors in the regulation of pain threshold with a photoactivable mGlu5 receptor negative allosteric modulator

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Abstract: The mGlu5 receptor is expressed at different levels of the pain neuraxis and its activation contributes to the development of nociceptive sensitization underlying chronic pain (reviewed by Montana and Gereau, Curr. Pharm. Biotechnol., 2011). Selective negative allosteric modulators (NAMs) of mGlu5 receptors have consistently shown analgesic activity in experimental animal models of inflammatory and neuropathic pain. However, the precise anatomical site of action of these drugs within the pain neuraxis remains to be determined. One way to address this question is to locally inject mGlu5 receptor NAMs in different stations of the pain neuraxis. However, the validity of this approach is limited by the diffusion of the drugs to neighbour regions, and the difficulty to perform accurate dose-response studies. An alternative approach is to systemically inject photoactivable drugs, which are activated by light irradiation specifically delivered into discrete brain region. This allows to control drug activity in specific localizations and time intervals. Here, we used a caged derivative of the mGlu5 receptor NAM, raseglurant (compound, JF-NP-026), which is activated by light at a wavelenght of 405 nm, in two mouse models of pain: (i) the formalin model of inflammatory pain; and (ii) the chronic constriction injury (CCI) model of neuropathic pain. In both models, JF-NP-026 and raseglurant were injectd i.p. at the dose of 10 mg/kg. Light was delivered by means of optic fibers bilaterally implanted in the ventrobasal thalamus. Systemic injection of raseglurant was higly effective in reducing nocifensive behavior in the first and second phase of the formalin test, regardless of light irradiation. In contrast, systemic injection of JF-NP-026 caused analgesia exclusively following light irradiation in the thalamus. The analgesic activity of the photoactivable compound was more ramarkable in the second phase of the formalin test, which reflects the development of central sensitization. Similar data were obtained in mice developing neuropathic pain, in which systemic injection of JF-NP-026 caused analgesia only after light irradiation in the thalamus at 7 and 14 days after CCI of the sciatic nerve. These findings show for the first time that thalamic mGlu5 receptors are targeted by mGlu5 receptor NAMs in the induction of analgesia.

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Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 587.05/E30

Topic: B.03. G-Protein Coupled Receptors

Title: Role of group II metabotropic glutamate receptors in methamphetamine behavioural sensitization in mice

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Abstract: Mechanisms of neuroadaptation of glutamatergic and dopaminergic transmission lie at the core of drug addiction. These mechanisms are modulated by mGlu2 and mGlu3 receptors, *via* the regulation of neurotransmitter release and other mechanisms, and pharmacological activation of mGlu2/3 receptors is reported to reduce the rewarding effect of commonly abused drugs and inhibit the reinstatement of drug seeking behavior. Therefore, mGlu2 and mGlu3 receptor ligands are considered as candidate drugs in the treatment of drug addiction. A main role for mGlu2 receptors in drug abuse was hypothesized on the basis of data obtained with mGlu2 receptor knockout mice undergoing cocaine sensitization (Morishima et al., Proc. Natl. Acad. Sci. USA, 102:4170-5, 2005). Here, we studied sensitization to motor effects of the psychostimulant, methamphetamine (METH), in mice lacking either mGlu2 or mGlu3 receptors and their wild-type counterparts. Mice were treated with METH (1 mg/kg) daily for 5 days. Motor behavior was assessed for 1 hour after METH or saline injection at day 1 and 5 of treatment, as well as after 6 days of withdrawal in response to an additional METH challenge (day 11). In wild-type mice, stimulation of locomotor activity by methamphetamine increased with time and was significantly higher at day 11. mGlu3 receptor knockout mice showed a greater motor response to METH, with sensitization being already significant at day 5. Interestingly, mGlu2 receptor knockout mice showed a blunted locomotor response to METH at day 1, but then locomotor sensitization was robust at both day 5 and day 11. All mice were killed at the end of the behavioral session (day 11) for measurements of METH-induced activation of the MAP kinase pathway in the nucleus accumbens (NAc) and other brain regions. Unexpectedly, METH was able to enhanced ERK1/2 phosphorylation in the NAc of wild-type mice, but not in the NAc of mGlu2 or mGlu3 receptor knockout mice. These findings suggest that mGlu2 receptors are required for the acute motor response to METH, and that both receptor subtypes contribute to mechanisms of behavioral sensitization.

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Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 587.06/E31

Topic: B.03. G-Protein Coupled Receptors

Title: Pharmacological evidence for a functional cross-talk between mGlu5 and mGlu3 receptors

Authors: L. DI MENNA[1], L. IACOVELLI[2], C. W. LINDSLEY[3], M. CANNELLA[1], T. IMBRIGLIO[1], M. ORLANDO[2], R. VERHAEGHE[1], J. MAIRESSE[4], P. GRESSENS[4,5], G. BATTAGLIA[1], J. P. CONN[3], *V. BRUNO[2,1], F. NICOLETTI[2,1];
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Abstract: Activation of polyphosphoinositide (PI) hydrolysis by the group-I metabotropic glutamate (mGlu) receptor agonist 3,5-dihydroxyphenylglycine (DHPG), in rat and mouse cortical and hippocampal slices was amplified by the mGlu2/3 receptor agonist (1R,4R,5S,6R)-4-amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid (LY379268). This potentiation was seen at all developmental ages with the exeption of the first 7-8 days of postnatal life in which the stimulation of PI hydrolysis by DHPG was too high to be further enhanced by LY379268. At this age, however, LY379268 could stimulate PI hydrolysis on its own, albeit to a small extent. All effects of LY379268 disappeared in cortical slices prepared from mGlu3 or mGlu5 receptor knockout mice, but persisted in slices prepared from mGlu2 receptor knocout mice or from *crv4* mice lacking mGlu1 receptors. Interestingly, DHPG-stimulated PI hydrolysis was halved in cortical slices from mGlu3 receptor knockout mice suggesting that endogenous activation of mGlu3 receptors is permissive for mGlu5 receptor-stimulated PI hydrolysis. The existence of a functional interaction between mGlu3 and mGlu5 receptors was supported by data obtained with the selective mGlu3 receptor negative allosteric modulator (NAM), VU0650786. Treatment of mouse cortical slices with VU0650786 dose-dependently reduced DHPG-stimulated PI hydrolysis in cortical slices from mice at postnatal day (PND) 7/8. In slices prepared from PND14/15 mice, compound VU0650786 abrogated the enhancing effect of LY379268 on DHPG-stimulated PI hydrolysis. The interaction between LY379268 and DHPG was also abolished by the mGlu5 receptor NAM, MPEP, but not by the mGlu1 receptor NAM,

JNJ16259685. An interaction between mGlu3 and mGlu5 receptors was also demonstrated in HEK-293 cells where it appeared to be mediated by the βγ subunits of Gi/o proteins. Experiments are ongoing to determine in which cell type (neurons, astrocytes, oligodendrocytes, microglia) the interaction between native mGlu3 and mGlu5 receptors takes place, and what is the functional relevance of this interaction during development and in the adult life.

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Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 587.07/E32

Topic: B.03. G-Protein Coupled Receptors

Title: Targeting mGlu1 receptors in the treatment of retinal degeneration

Authors: M. ROMANO[1], D. BUCCI[1], F. LIBERATORE[1], L. DI MENNA[1], M. MADONNA[1], R. GRADINI[2,1], V. BRUNO[2,1], G. BATTAGLIA[1], *F. NICOLETTI[2,1];
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Abstract: Glaucoma is chronic neurodegenerative disorders characterized by progressive degeneration of retinal ganglion neurons, in which age and increased intraocular pressure (IOP) are major risk factors. Drugs that lower IOP are standard in the glaucoma treatment and there are no neuroprotective drugs that shows clinical efficacy. Thus, the identification of a new therapeutic target in the treatment of glaucoma is an important medical need. We found recently that stimulation of polyphosphoinositide (PI) hydrolysis by excitatory aminoacid in the retina is exclusively mediated by metabotropic glutamate mGlu1 receptors (Romano et al., Neurochem. Res., 2016). These receptors are involved in neurodegeneration/neuroprotection mechanisms because their activation stimulates Ca^{2+} release from intracellular stores. Depending of the extend and the dynamic of Ca^{2+} release as well as on the cellular contest receptor activation by either enhance or attenuate neurodegeneration. We are examining the role of mGlu1 receptors in retinal neurodegeneration by using selective mGlu1 receptor ligands and mutant mice lacking mGlu1 receptors (*crv*4 mice). We are using two models to investigate retinal degeneration: (i) systemic injection of monosodium glutamate in neonatal mice; and, (ii) DBA/2J mice which develop an increase of IOP after five months of age. Mice are systemically treated with either the selective mGlu1 receptors NAM, JNJ16259685 or the selective mGlu1 receptors PAM, Ro 07-11401. In DBA/2J mice and their controls, treatment was performed by subcutaneously

implanted osmotic minipump during either the pre-symptomatic phase (from five to seven months of age) and symptomatic phase (from seven to nine months of age). Current data obtained with DBA/2J mice treated with JNJ16259685 for two months started in pre-symptomatic phase suggest that mGlu1 receptors might represent a potential therapeutic target in the drug treatment of glaucoma and other disorders characterized by retinal degeneration.

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Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 587.08/E33

Topic: B.03. G-Protein Coupled Receptors

Support: IISER Mohali

Title: Role of ubiquitination in glutamate receptors trafficking

Authors: ***R. GULIA**, R. SHARMA, S. BHATTACHARYYA;
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Abstract: Glutamate is a major excitatory neurotransmitter in the central nervous system (CNS). Glutamate receptors which bind to glutamate are categorized into two groups: ionotropic glutamate receptors (ion channels) and metabotropic glutamate receptors (GPCRs). Group I metabotropic glutamate receptors (mGluRs) consists of two members viz., mGluR1 and mGluR5. They are predominantly localized at the post-synaptic side are key players in the process of neuronal development as well as in synaptic plasticity in the CNS. These receptors are positively coupled to Gq pathway. Accurate temporal and spatial localization of these receptors at postsynaptic surface is crucial for normal signaling, and their spatiotemporal localization is maintained by trafficking of the receptor. Trafficking also play crucial role(s) in the regulation of these receptors. Thus, studies of the trafficking of these receptors are of utmost importance. Furthermore, these receptors upon activation result in the endocytosis of AMPA receptors, leading to mGluR-dependent Long-term depression (mGluR-LTD). We are interested in investigating the cellular and molecular mechanisms that govern the group I mGluR trafficking and it effect on mGluR-mediated AMPAR endocytosis. Our data suggest that ubiquitination plays critical role in the trafficking of these receptors. We have found that these receptors get ubiquitinated upon binding with the ligand and subsequently get endocytosed. We further

demonstrated that lysine residue present at the C-terminus of mGluR1 plays critical role in the endocytosis of mGluR1. In addition, E3 ligase, Siah-1A was found to be involved the ubiquitination of mGluR1. Acute knockdown of Siah-1A also enhanced group1 mGluR mediated AMPAR endocytosis in primary neurons.

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Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

Location: Halls B-H

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Topic: B.03. G-Protein Coupled Receptors

Support: NIDA Grant R01DA033342

P50DA015369)

T32 Grant DA007288)

Title: Acute stress exposure mediates circuit-specific, neuroadaptations in glutamate inhibition in VTA-dopaminergic neuron

Authors: *J. PARRILLA-CARRERO, P. GOSWAMEE, C. BAILES, B. PAVLINCHAK, A. RIEGEL;
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Abstract: Environmental stressors contribute to the reinforcing effects of drugs of abuse. Stress triggers the release of corticotropin-releasing factor (CRF) in the brain, which potentiates glutamatergic signaling at dopamine (DA) neurons in ventral tegmental area (VTA) via ionotropic receptors. However, glutamate also recruits inhibition in VTA neurons via activation of postsynaptic metabotropic glutamate receptors (mGluRs). Activation of mGluRs mobilizes intracellular calcium stores to activate inhibitory small-conductance calcium-activated potassium (sK) channels. This mGluR/sK inhibition impacts DA neuron firing patterns and presumably affects the processing of salient information in VTA-projection areas, such as the nucleus accumbens (NAc). Here we investigate how stress-associated CRF impacts mGluR/sK inhibition in dopamine neurons. Because the NAc core and shell subregions likely regulate value coding and prediction monitoring differently, we distinguished between core projecting (VTA◊Core) and shell projecting (VTA◊Shell) VTA neurons with the use of fluorescent microspheres. Three weeks after fluorescent microsphere injection, rats (male, P70) were exposed to filter paper wetted with either alcohol vehicle (control) or TMT (1%). Brain slices were made either 2hrs or

24 hrs later. Inhibitory sK channel currents were measured using whole-cell patch clamp electrophysiology, in response to synaptic stimulation or flash photolysis to uncage intracellular calcium (NP-EGTA). CRF (200 nM) or forskolin (1 uM), an activator of adenylyl cyclase, was applied by superfusion to stimulate stress hormone related signaling. In control animals, sK currents were stronger (larger amplitude and slower decay kinetics) in ***VTA Core*** than ***VTA shell*** projecting DA neurons. A single TMT(24hr) exposure reduced the maximal (basal) sK current in ***VTA Core*** cells and appeared to inactive/desensitize the CRF or forskolin facilitation of sK currents as observed under control or TMT(2hr) conditions. In contrast, in the ***VTA shell*** cells possessing comparatively weak (basal) glutamate inhibition, TMT (24hr) treatment potentiated (basal) IPSCs and simultaneously occluded the CRF or forskolin facilitation. Both populations of cells displayed sK-dependent inhibitory spontaneous miniature outward currents (SMOCs) following TMT(24hrs) treatment, further corroborating an action of stress on glutamate inhibition. Thus, TMT stress alters glutamate inhibition in the VTA, inducing a loss of function in ***VTA Core*** circuitry and a gain of function in ***VTA shell*** circuitry. Such changes are predicted to modulate DA release during reward-predictive cues.

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Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

Location: Halls B-H

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Topic: B.03. G-Protein Coupled Receptors

Support: NIH Fellowship NS089198

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Title: Dynamic regulation of $GABA_B$Rs by neural activity and phosphatase signaling

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Abstract: Activation of metabotropic GABA type-B receptors ($GABA_BRs$) mediates slow, sustained neural inhibition and critically limits the damage caused by excess excitation, such as occurs during seizure or stroke. $GABA_BRs$ comprise heterodimers of R1 (GABA-binding) and R2 ($G_{i/o}$-binding) subunits. They act presynaptically to block neurotransmitter release via inhibition of voltage-gated Ca^{2+} channels and postsynaptically to hyperpolarize neuronal membrane potential via activation of inwardly rectifying K^+ (GIRK) channels. Our lab has previously identified a bi-phasic regulation of $GABA_BRs$ by activation of NMDA-type glutamate receptors (NMDARs) and increases in intracellular Ca^{2+}. Initially, 5'AMP-dependent protein kinase (AMPK)-mediated phosphorylation of Ser-783 of the R2 subunit increases, in turn stabilizing $GABA_BRs$ at the plasma membrane, but during prolonged or excitotoxic stimulation, protein phosphatase 2A (PP2A)-dependent dephosphorylation of Ser-783 becomes predominant and $GABA_BRs$ are endocytosed and degraded. This NMDAR-mediated impairment of $GABA_BR$ signaling may exacerbate excitotoxic neuronal death, and has also been observed in response to chronic stress and psychostimulant administration. Therefore, preventing R2 Ser-783 dephosphorylation may represent a promising therapeutic intervention for multiple neurological and psychiatric disorders. Our preliminary data indicate that this regulation of R2 is engaged in hippocampal slices in response to NMDA stimulation, oxygen-glucose deprivation (OGD) – an ischemic stroke model – and exposure to Mg^{2+}-free conditions – a seizure model, as well as *in vivo* in the hippocampi of mice subjected to kainate-induced seizures. Ongoing experiments investigate whether these effects are altered in knock-in mice bearing either constitutively de-phosphorylated (Ser to Ala; S783A) or putatively phospho-mimetic (Ser to Asp; S783D) mutations of R2 Ser-783. Additional experiments combining affinity purification and mass spectrometry approaches seek to identify the motif in the R1 $GABA_BR$ subunit to which PP2A binds and the PP2A isoforms that participate in this interaction. Disruption of R1 binding to PP2A is predicted to ameliorate the dephosphorylation of R2 Ser-783 observed in various models of neuropathology, which may guide the development of novel therapeutics.

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Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

Location: Halls B-H

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Program#/Poster#: 587.11/E36

Topic: B.03. G-Protein Coupled Receptors

Support: MOST 102-2628-B-002-024-MY3

Title: Autoregulation of $GABA_B$ receptor signaling through with extracellular signal-regulated kinase activation in rat locus coeruleus

Authors: *R.-N. WU[1], M.-Y. MIN[1], H.-W. YANG[2];
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Abstract: The metabotropic $GABA_B$ receptor ($GABA_BR$) plays important roles in regulating neuronal excitability in the brain. $GABA_BR$ is well known to exert both pre- and postsynaptic inhibition through inhibiting voltage-gated Ca^{2+} channel and activating K^+ (GIRK) channel, respectively. Beside these effects, here we found that $GABA_BR$ activation caused an increase in phosphorylated extracellular signal- regulated kinase (pERK) level in locus coeruleus (LC), which consists of noradrenergic neurons and plays divers roles in behavior. Using western-blot analysis, LC tissue punched out form brain slices bathed in 50 μM baclofen showed an increase in pERK1 and pERK2 by, respectively, 23±7% and 35±13% ($P<0.05$ for both cases, N=6), compared to tissue from slices bathed in normal medium. This effect was specific to $GABA_BR$ activation as it was not observed in LC tissue from slices bathed with baclofen and 10 μM CGP54626, a $GABA_BR$ blocker. The activated ERK seems paly a role in stabilizing $GABA_BR$ signaling. Bath application of baclofen for 15 min induced a CGP54626 sensitive outward current (I_{Bac}) in LC neuron (Vm -70 mV) that underwent slow and partial desensitization. In slices pretreated with or bathed in ERK blockers, U0126 or FR180204, I_{Bac} showed a faster and more prominent desensitization; the half-decay time of I_{Bac} reduced to 51 ± 8% of control for U0126 treatment ($p<0.01$, N=7) and to 57 ± 7% for FR180204 ($p<0.05$, N=7). Since $GABA_BR$ has been shown to mediate a tonic inhibition, defined as rebound increase in firing rate (FR) upon $GABA_BR$ blocker application, of LC neurons, we tested the effect of ERK-dependent desensitization of I_{Bac} on it. In slice pretreated with baclofen for 30 min, FR of LC neurons increased to 239 ± 54% of baseline upon CG54626 application (N=8); it while reduced to 134 ± 10% as for pretreatment with baclofen plus U0126 and to 120 ± 5% as for pretreatment with baclofen plus FR18024 (N=8). Together, the above results show that $GABA_BR$ activation recruits ERK-signaling pathway for an autoregulation that prevents $GABA_BR$ from quick desensitization, therefore maintains tonic inhibition, an important mechanism for tuning FR of LC neurons.

Disclosures: **R. Wu:** None. **M. Min:** None. **H. Yang:** None.

Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 587.12/E37

Topic: B.03. G-Protein Coupled Receptors

Support: SNSF Grant 31003A_143373 / 1

Title: The nogo-$A^{-/-}$ mouse, a model for schizophrenia, exhibits altered hippocampal CA3 function and mglu3 receptor expression

Authors: ***S. BERRY**[1], O. WEINMANN[1], A.-K. FRITZ[2], D. WOLFER[2], M. E. SCHWAB[3], U. GERBER[1], J. STER[4];
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Abstract: Nogo-A is a membrane protein well established as an inhibitor of neurite outgrowth in the CNS that regulates the formation of neuronal circuits during development, but curtails axonal regeneration after CNS injury. Recent evidence from human genetic analyses and experiments in animals implicates Nogo-A dysfunction in certain forms of schizophrenia. Using electrophysiology, microscopy and behavioral tasks in transgenic mice, we characterized the roles of Nogo-A in the hippocampus. Patch clamp recordings from CA3 hippocampal pyramidal cells revealed that the absence of Nogo-A caused a significant increase in spontaneous excitatory activity. In addition, mGlu3 metabotropic glutamate receptors, which can exhibit mutations in schizophrenia, were down-regulated specifically in the CA3 region of Nogo-$A^{-/-}$ mice. This reduction in mGlu3 receptors was accompanied by abnormal CA3 theta oscillations manifesting as a decrease in incidence and frequency, as well as a shift in polarity from inhibitory to predominantly excitatory. These disruptions to CA3 function altered processing of reference frames during spatial navigation in that Nogo-$A^{-/-}$ mice exhibited a greater dependence on global as opposed to local reference frames. These results show that the absence of Nogo-A disrupts hippocampal activity by increasing CA3 excitation and reducing the expression of mGlu3 receptors. Previous data suggested that alterations in either protein can contribute to psychosis. While there is a long list of candidate genes for psychiatric diseases, which typically display multifactorial etiologies, in most cases potential interactions have not been determined. The characterization of a functional link between Nogo-A and mGlu3 receptors, may thus provide novel insights into the underlying pathophysiology of schizophrenia.

Disclosures: **S. Berry:** None. **O. Weinmann:** None. **A. Fritz:** None. **D. Wolfer:** None. **M.E. Schwab:** None. **U. Gerber:** None. **J. Ster:** None.

Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

Location: Halls B-H

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Topic: B.03. G-Protein Coupled Receptors

Support: NIH Grant R01 MH062646

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PhRMA Foundation Postdoctoral Fellowship in Pharmacology/Toxicology

Title: Molecular pharmacology of $mGlu_2$ allosteric modulators.

Authors: *D. E. O'BRIEN[1], A. J. CROSS[3], S. WESOLOWSKI[3], J. BERGARE[4], C. S. ELMORE[4], H. P. CHO[2], P. J. CONN[2];
[1]Pharmacol., [2]Vanderbilt Univ., Nashville, TN; [3]AstraZeneca Neurosci. Innovative Medicines, Cambridge, MA; [4]AstraZeneca Drug Safety & Metabolism, Molndal, Sweden

Abstract: Metabotropic glutamate receptor 2 ($mGlu_2$) offers a promising neurotherapeutic target for the treatment of various brain disorders. Previous work demonstrates that $mGlu_2$ positive allosteric modulators (PAMs) exhibit efficacy in preclinical models of schizophrenia, pain, addiction, epilepsy, and anxiety disorders. Likewise, dual $mGlu_{2/3}$ negative allosteric modulators (NAMs) display cognitive enhancing properties and efficacy in preclinical models of depression. Recently, medicinal chemistry efforts directed at specifically targeting $mGlu_2$ have successfully developed several chemically distinct $mGlu_2$ PAMs and NAMs. In order to better understand the pharmacology and binding modes of these novel $mGlu_2$ allosteric modulators, we characterized their *in vitro* binding to recombinant rat $mGlu_2$ ($RmGlu_2$) using competition binding experiments with an $mGlu_2$ PAM radioligand. Additionally, we characterized their functional properties using *in vitro* assays in wildtype and mutant $RmGlu_2$ that, along with the competition binding experiments, aimed to understand if these novel allosteric modulators act through a common binding site or distinct sites on $mGlu_2$. Finally, we performed further experiments to better understand how $mGlu_2$ agonists modulate the affinity of various $mGlu_2$ allosteric modulators. Taken together, these studies will improve our understanding of both the pharmacology and binding modes of $mGlu_2$ allosteric modulators.

Disclosures: **D.E. O'Brien:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca. **A.J. Cross:** A. Employment/Salary (full or part-time): AstraZeneca. **S. Wesolowski:** A. Employment/Salary (full or part-time): AstraZeneca. **J. Bergare:** A. Employment/Salary (full or part-time): AstraZeneca. **C.S. Elmore:** A. Employment/Salary (full or part-time): AstraZeneca. **H.P. Cho:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca. **P.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; AstraZeneca. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Holds patents on mGlu allosteric modulators.

Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 587.14/F1

Topic: B.03. G-Protein Coupled Receptors

Support: NRF-2011-0011694

the Brain Korea 21 PLUS

Seoul National University Hospital

Title: Post-translational modifications regulate mGluR7 trafficking and signaling

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Abstract: The metabotropic glutamate receptors (mGluRs) are seven membrane-spanning proteins that are linked via G-proteins to intracellular signaling cascades. Among eight family members of mGluRs, mGluR7 is a predominant group III mGluR that is highly expressed at the presynaptic active zone and acts as an auto-regulatory receptor to limit neurotransmitter release. Like many GPCRs, mGluR7 undergoes constitutive and agonist-dependent endocytosis. Besides phosphorylation, GPCR endocytosis can be tightly regulated by post-translational modifications of lysine residues such as SUMOylation and ubiquitination. Previously we showed that SUMO modification at Lys889 residue of mGluR7 modulates constitutive and agonist-dependent mGluR7 trafficking. As mGluR7 harbors eight lysine residues in the cytoplasmic domain and

additional four lysine residues in the intracellular loops, we now have mapped the sites of ubiquitination that may affect mGluR7 trafficking and turnover. Given the importance of the ubiquitin-proteasome system in neurodegenerative and neuropsychiatric disorders, mechanisms underlying mGluR7 ubiquitination will provide insights into the functional role of mGluR7 in neurological diseases as well as in learning and memory.

Disclosures: **S. Lee:** None. **S. Han:** None. **H. Lee:** None. **S. Kim:** None. **S. Park:** None. **Y. Suh:** None.

Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

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Program#/Poster#: 587.15/F2

Topic: B.03. G-Protein Coupled Receptors

Support: NIDA Grant DA032701

Title: Neurotensin depresses gaba type-b receptor-mediated neurotransmission in the substantia nigra through pre- and postsynaptic mechanisms.

Authors: ***C. TSCHUMI**[1], M. J. BECKSTEAD[2];
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Abstract: Midbrain dopamine neurons play physiological roles in many processes including reward learning and motivated behavior. Conversely, dysfunction of these neurons is implicated in disorders such as schizophrenia and drug abuse. Midbrain dopamine neurons are tonically inhibited by γ-Aminobutyric acid (GABA)ergic inputs from multiple brain regions. Neurotensin (NT) is a neuropeptide which modulates midbrain dopamine neuron excitability through multiple mechanisms, including a decrease of GABA mediated inhibition of dopamine neurons. However, it is not known if NT acts post-synaptically on GABA type-B receptor signaling, pre-synaptically at GABA terminals to alter GABA release, or through a combination of synaptic mechanisms. Here we utilize whole cell patch-clamp electrophysiology of dopamine neurons in brain slices to show that neurotensin acts both pre- and postsynaptically to decreases GABA type-B receptor-mediated currents in the substantia nigra in mice. Bath perfusion of neurotensin produced a sustained depression of GABA type-B receptor-mediated currents that was more pronounced when GABA was released endogenously (due to electrical stimulation) compared to when GABA was directly applied to the cell (via iontophoresis). GABA type-B receptor mediated currents exhibited paired pulse depression when currents were elicited with electrical stimulation, but not GABA iontophoresis. Bath application of neurotensin increased the paired

pulse ratio with electrical stimulation but had no effect on paired pulse currents elicited by GABA iontophoresis, providing further evidence neurotensin modulates GABA release. As NT is an endogenous peptide present at high levels in the midbrain, determining the mechanism of action by which NT alters midbrain dopamine neuron excitability is a crucial step in understanding the importance of NT in dopamine mediated behavior and related disorders.

Disclosures: C. Tschumi: None. **M.J. Beckstead:** None.

Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

Location: Halls B-H

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Program#/Poster#: 587.16/F3

Topic: B.03. G-Protein Coupled Receptors

Title: Quantifying cns m1 muscarinic acetylcholine receptor modulation using an *In vivo* ip1 accumulation assay

Authors: *M. POPIOLEK[1], D. NGUYEN[2], V. REINHART[1], J. EDGERTON[1], J. HARMS[1], S. M. LOTARSKI[1], S. J. STEYN[2], J. E. DAVOREN[3], S. GRIMWOOD[1];
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Abstract: The rationale for M_1-selective muscarinic acetylcholine receptor (mAChR) activators for the treatment of cognitive impairment associated with psychiatric and neurodegenerative disease is well established in the literature. M_1 activation by a positive allosteric modulator (PAM) is a new approach for achieving subtype selectivity. Here, we present a correlation between mouse amphetamine stimulated locomotor activity of 5 CNS penetrant M_1-subtype selective PAM-agonists with in-vivo increases in mouse striatal inositol phosphate (IP_1) levels. IP_1 accumulation in the striatum was measured from microwaved brain tissue 2 hours after dosing mice with an M_1 mAChR PAM and LiCl and quantified as a multiple over baseline effects. The IP_1 accumulation assay is shown to be a robust biochemical measure of M_1 activation that correlates well with in-vivo pharmacodynamic activity from the amphetamine stimulated locomotor activity assay. Pharmacokinetic-pharmacodynamic modeling of the IP_1 response was used to examine the relationship between exposure and in-vivo IP_1 accumulation, permitting rank ordering of compounds by their ability to potentiate M1 in-vivo under an endogenous level of acetylcholine.

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Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

Location: Halls B-H

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Program#/Poster#: 587.17/F4

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NHMRC Grant APP1084775

Title: Evaluation of metabotropic glutamate receptor 5 allosteric ligands in recombinant and native tissues: evidence for biased agonism and modulation.

Authors: *K. SENGMANY;
Univ. of Nottingham, Nottingham, United Kingdom

Abstract: Emerging evidence implicates altered glutamatergic neurotransmission in various CNS disorders, with the metabotropic glutamate receptor subtype 5 ($mGlu_5$) representing a particularly promising new therapeutic target. Design of small molecule $mGlu_5$ allosteric ligands offers the advantage of spatial and temporal fine-tuning of endogenous agonist activity, allowing for increased selectivity, reduced adverse effects and improved clinical outcomes. Positive allosteric modulators (PAMs) enhance, whereas negative allosteric modulators (NAMs) inhibit, $mGlu_5$ activity. Recently, we have shown that $mGlu_5$ allosteric ligands classified as 'PAMs' or 'PAM-agonists' for intracellular Ca^{2+} (iCa^{2+}) mobilization display biased agonism, relative to the orthosteric agonist, DHPG, when comparing iCa^{2+} mobilization with $mGlu_5$-mediated ERK1/2 phosphorylation and IP_1 accumulation in both recombinant cells and mouse cortical neurons. Further, VU0360172 exhibited different degrees of cooperativity with DHPG in a signalling pathway-dependent manner. To gain a better understanding of the full scope of $mGlu_5$ allosteric ligand actions, therefore, it is apparent that such rigorous pharmacological profiling also needs to be extended to $mGlu_5$ ligands currently classified as 'NAMs'. Here, we describe the signalling profile of diverse $mGlu_5$ allosteric NAMs, at iCa^{2+}, IP_1 and pERK1/2 receptor signalling endpoints. All NAMs were inverse agonists for IP_1 accumulation in HEK293A expressing $mGlu_5$, however inverse agonism was not apparent in cortical neurons, indicating context-dependent activity. The rigorous profiling of $mGlu_5$ allosteric ligands was further extended to native peripheral cell types. Previous studies suggest that $mGlu_5$ can modulate pain responses, as evidenced by glutamate enhancement of capsaicin activity and sensitised pain responses both *in vivo* and *in vitro* (1,2). Thus, we assessed the modulatory activity of select $mGlu_5$ allosteric

ligands on the transient receptor potential cation channel, TRPV1, in rat dorsal root ganglia. In summary, our work aims to provide a greater appreciation of the pharmacology of $mGlu_5$ allosteric ligands in both recombinant and native cell systems – laying the groundwork for rational design and discovery of drugs with greater clinical efficacy and reduced adverse effects.
(1) Masuoka T *et al* (2015). *Br J Pharmacol* **172**(4): 1020-1033.
(2) Walker K *et al* (2001). *Neuropharmacology* **40**(1): 10-19.

Disclosures: K. Sengmany: None.

Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

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Topic: B.03. G-Protein Coupled Receptors

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ANR-15-CE18-0020-01

MNRT

Title: A new positive allosteric modulator specific of subtype 2 metabotropic glutamate receptors ($mglur_2$) modulates native receptor activity in the rat hippocampus

Authors: *S. BOSSI[1], P. SCHOLLER[2], D. NEVOLTRIS[2], X. ROVIRA[2], E. DUPUIS[3], D. BATY[4], J.-P. PIN[2], P. RONDARD[2], H. DANIEL[1], H. MCLEAN[1];
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Abstract: Metabotropic glutamate receptors (mGluRs) are G protein coupled receptors abundantly found in the central nervous system. Expressed at both pre- and postsynaptically, these receptors can strongly influence synaptic transmission. As such, mGluRs are a potential

target for therapeutic treatment of diverse neurological disorders. mGluRs are dimeric receptors with a large extracellular glutamate binding domain (the venus flytrap domain) whose structure is highly conserved among the 8 subtypes of mGluRs. As such, developing molecules that are specific to one particular subtype of mGluR is challenging issue. An innovative pharmacological tool with which to study these receptors resides in single-chain antibodies (nanobodies) raised in *camelidae* that are directed to specific conformations of a specific subtype of mGluR. We studied the effect of a high affinity nanobody, developed as a **P**ositive **A**llosteric **M**odulator (PAM) of $mGluR_2$, on the activity of this receptor in an *ex vivo* model system, the hippocampal slice preparation.

In the hippocampus $mGluR_2$ are found on Mossy Fiber (MF) terminals where their activation reduces glutamate release at the MF-CA3 pyramidal cell synapse by negatively regulating calcium entry and exocytosis. Using photometric calcium imaging techniques and pharmacological tools, we first verified the presence of $mGluR_2$ on MF terminals. MF were stimulated every 30 seconds, with a single 100-Hz train of 5 electrical stimuli, through a saline-filled glass electrode positioned in the mossy fiber tract. Stimulation evoked presynaptic calcium transients were reversibly depressed by bath application of the group II mGluR orthosteric ligand DCG IV (100 nM). Subsequent application of high affinity nanobodies specifically directed against $mGluR_2$ induced a positive modulation of the orthosteric ligand affinity. Both the overall amplitude and the time course of DCG IV effects were significantly accentuated in the presence of micromolar concentrations of the nanobody. Furthermore, this enhancement was stronger than that observed with a well-known $mGluR_2$ positive allosteric modulator (PAM), LY487379 (50microM).

Our results suggest that site directed nanobodies function as efficient PAMs for mGluRs. This new pharmacological tool is extremely attractive for studying the molecular basis of metabotropic glutamate receptor activity, and should allow us to further dissect the physiological role of different subtypes of mGluRs in the brain.

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Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

Location: Halls B-H

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Program#/Poster#: 587.19/F6

Topic: B.03. G-Protein Coupled Receptors

Support: This work is supported by the NIAAA Division of Intramural Clinical and Biomedical Research.

NIGMS PRAT Fellowship to K.A.J.

Title: Metabotropic glutamate receptor 2 modulates thalamostriatal neurotransmission.

Authors: *K. A. JOHNSON[1], D. M. LOVINGER[2];
[1]Lab. for Integrative Neurosci., NIH, Rockville, MD; [2]NIAAA/NIH, Rockville, MD

Abstract: The striatum plays important roles in motor control and action learning. The activity of striatal projection neurons is controlled by inputs from several brain regions, and the strength of these inputs can be modulated by presynaptic G protein-coupled receptors (GPCRs). Most previous efforts to evaluate GPCR-mediated modulation of glutamatergic transmission in the striatum have attributed changes in electrically-evoked transmission to modulation of corticostriatal circuits. However, the contributions of excitatory inputs originating in the thalamus, which represent almost half of excitatory inputs to medium spiny neurons (MSNs), are frequently overlooked. Activation of group II metabotropic glutamate receptors ($mGlu_2$ and $mGlu_3$) is known to produce a strong inhibition of electrically-evoked glutamatergic transmission onto striatal medium spiny neurons (MSNs). To evaluate the input specificity of the $mGlu_{2/3}$-mediated modulation of striatal glutamatergic transmission, we used a viral strategy to express Channelrhodopsin-2 (ChR2) in thalamostriatal projection neurons of C57Bl/6J mice. 3-6 weeks after virus injection, coronal slices containing the striatum were prepared, and optically-evoked thalamostriatal excitatory postsynaptic currents (oEPSCs) were recorded from MSNs in the dorsolateral striatum. Bath application of the group II mGlu receptor agonist LY379268 (100 nM, 5 min) produced a robust reduction of oEPSC amplitude (peak depression 27.4±6.0% of baseline). Inhibition of oEPSCs by LY379268 was long lasting, persisting for at least 45 minutes after the onset of drug application (oEPSC amplitude 44.5±5.0% of baseline 40-45 minutes after LY379268 application). Interestingly, inhibition of optically-evoked corticostriatal oEPSCs by LY379268 was less robust. To identify which receptor subtype(s) contribute to the inhibition of thalamostriatal transmission, we evaluated the effect of the $mGlu_2$ agonist/$mGlu_3$ antagonist LY395756 (10 μM, 5 min). LY395756 produced a strong, reversible inhibition of oEPSC amplitude (peak depression 22.3±2.5% of baseline), suggesting a major role for $mGlu_2$ in the modulation of thalamostriatal transmission. Conversely, a negative allosteric modulator of $mGlu_3$ failed to block the effect of LY372968. These findings add to our limited knowledge of the mechanisms regulating the thalamostriatal system and demonstrate the utility of optogenetics for the study of input-specific modulation of neurotransmission.

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Poster

587. Metabotropic Glutamate Receptors and $GABA_B$ Receptors

Location: Halls B-H

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Program#/Poster#: 587.20/F7

Topic: B.03. G-Protein Coupled Receptors

Support: R21 MH102548

Autism Speaks

rettsyndrome.org

Title: Metabotropic glutamate receptor 7 as a therapeutic target in MECP2-related disorders

Authors: *C. M. NISWENDER[1,3], R. G. GOGLIOTTI[1], N. M. FISHER[1], R. SENTER[1], R. W. GOULD[1], J. J. ADAMS[1], B. J. STANSLEY[1], A. G. WALKER[1], R. ZAMORANO[1], A. L. BLOBAUM[1], D. W. ENGERS[1], C. R. HOPKINS[2], C. W. LINDSLEY[2], C. K. JONES[1,3], Z. XIANG[1], P. J. CONN[1,3];
[1]Pharmacol. and Vanderbilt Ctr. for Neurosci. Drug Discovery, [2]Pharmacology, Chem. and Vanderbilt Ctr. for Neurosci. Drug Discovery, Vanderbilt Univ., Nashville, TN; [3]Vanderbilt Kennedy Ctr., Nashville, TN

Abstract: Mutation or duplication of the Methyl CpG Binding Protein 2 (*MECP2*) gene results in the disorders Rett syndrome (RS) and *MECP2* Duplication syndrome (MDS), respectively. Based on their reciprocal molecular origins, RS and MDS might be viewed as opposing diseases with distinct phenotypes; however, the striking overlap of some symptoms (e.g., respiratory phenotypes, autism, cognitive impairments, seizures) suggests that certain disease-relevant pathways may be similarly disrupted between the two disorders, pointing to a potentially paradoxical, yet shared, therapeutic strategy. We have found that the gene encoding one of the metabotropic glutamate receptors (mGlus), *GRM7*, is a transcriptional target of MeCP2. *GRM7* codes for the presynaptically-expressed $mGlu_7$ receptor, a regulator of both GABA and glutamate release. We have observed dramatic reductions in $mGlu_7$ expression in *Mecp2*-deficient mice, a model of RS, as well as an approximately 50-70% reduction in $mGlu_7$ protein levels in cortical and cerebellar samples from RS patients. Reduced $mGlu_7$ expression correlates with attenuated hippocampal long term potentiation (LTP) at the Schaffer Collateral-CA1 (SC-CA1) synapse in *Mecp2*-deficient mice, and potentiation of $mGlu_7$ using positive allosteric modulators (PAMs) can normalize LTP and rescue learning and memory deficits. These findings led us to the hypothesis that increased MeCP2 expression in the MeCP2-Tg1 model of MDS might result in increased $mGlu_7$ levels and function, suggesting that an $mGlu_7$ *negative* allosteric modulator (NAM) could be effective in reversing abnormalities. However, contrary to our hypothesis, we have now generated data indicating that disease progression does not occur on a

linear path in MDS model mice, but rather evolves as the disease progresses. While hippocampal $mGlu_7$ protein levels are similar to controls in 6-week-old mice, by 20 weeks of age, levels in MeCP2-Tg1 animals are reduced by approximately 50%. Consistent with this finding, an $mGlu_7$ *PAM* normalizes learning and memory responses in 20-week-old MeCP2-Tg1 mice. Remarkably, this is the same treatment strategy we utilized in *Mecp2*-deficient mice. These findings suggest that the biology underlying RS and MDS may merge over time, pointing to a potential common etiology. It will now be critical to understand the regulation of $mGlu_7$ levels and function longitudinally across these disease states to understand temporal and spatial changes that might occur during disease course to explore the therapeutic potential of $mGlu_7$ modulation in these two disorders.

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Poster

588. Potassium Channels II

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Topic: B.04. Ion Channels

Support: NIH NS036855

Howard Hughes Medical Institute

Title: Differential contribution of Kv1 channels to action potentials and firing patterns of defined primary sensory neuron subtypes

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Abstract: Mammalian neurons display a wide variety of action potential shapes and firing patterns, reflecting differences in the expression levels of different types of ion channels. We explored how differences in expression of voltage-dependent potassium channels in different types of primary sensory neurons are correlated with differences in firing patterns, focusing on Kv1 family channels. Using mouse genetic models in which defined types of mouse dorsal root ganglion neurons are identified by fluorescent labels, we made electrophysiological recordings from acutely-isolated cell bodies of defined neuronal types. The mRNA expression levels of

channels were also determined in each cell type using FACs purified neurons with next generation deep sequencing. In three different cell types corresponding to mechanoreceptive Abeta fibers, there was high expression of mRNA for Kv1.1 and Kv1.2 channel subunits, with little expression of other Kv1 family subunits. In these neurons, action potential clamp experiments showed that a major component of current during the action potential was carried by dendrotoxin-sensitive Kv1 potassium channels. The dendrotoxin-sensitive Kv1 current activated before the threshold of the action potential and peaked during the action potential falling phase. With maintained current injections of increasing size, most Abeta cell bodies fired only one or two action potentials under control conditions but could fire repetitively after Kv1 channels were blocked by dendrotoxin. In contrast to the Abeta fiber cell bodies, small-diameter neurons corresponding to non-peptidergic nociceptors expressed much lower levels of mRNA for Kv1.1 and Kv1.2 channels, and in these neurons there was only a small contribution of dendrotoxin-sensitive current to the repolarization of the action potential and dendrotoxin had little effect on the firing pattern, which was characterized by repetitive spiking at low and moderate frequencies. Thus, Kv1 channel expression determined by mRNA levels is much higher in DRG neurons corresponding to Abeta fibers than those corresponding to non-peptidergic nociceptors and mRNA levels are well-correlated with different functional contributions of Kv1 current to the firing properties of the neurons.

Disclosures: **P. Liu:** None. **Y. Zheng:** None. **D.D. Ginty:** None. **B.P. Bean:** None.

Poster

588. Potassium Channels II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 588.02/F9

Topic: B.04. Ion Channels

Support: MH 064711

NS 085330

Title: Cell-type specific activity-dependent regulation of potassium currents in CPG neurons

Authors: *D. SALLOUM[1], J. GOLOWASCH[2];
[1]NJIT, Newark, NJ; [2]Biol. Sci., New Jersey Inst. of Technol., Newark, NJ

Abstract: Regulation of K^+ currents is one of the most prominent mechanisms neurons utilize to alter their excitability in an activity-dependent manner. Many studies have shown activity-dependent changes in ionic currents in STG neurons (Golowasch et al 1999; Turrigiano et al

1995; Haedo & Golowasch 2006). In inferior cardiac (IC) neurons, for example, depolarizing current injections elicited a decrease in the high threshold K^+ current, I_{HTK}, and an increase in the transient K^+ current, I_A. We tested the hypothesis that different cell types within a single motor network regulate ionic current levels in response to patterned activity differently given their distinct intrinsic properties. We measured both I_{HTK} and I_A in pyloric dilator (PD), lateral pyloric (LP), and IC neurons before and after imposing up to two hours of patterned activity using two electrode voltage clamp. We drove the membrane potential of the neurons with three different activity profiles: (1) depolarizing stimuli with square pulses from -60mv to -10mv at a frequency of 1Hz, a patterned voltage fluctuation comparable to their endogenous activity (2) hyperpolarizing stimuli to range from -60mV to -110mV also at 1Hz (3) clamping to -60mV with no change over time. We measured currents every 30 minutes to test for changes in current amplitude assuming post-translational mechanisms are occurring. PD neurons exist as pairs within each ganglion and comprise the pacemaker kernel, giving this neuron a role in setting network frequency. Only when neurons were depolarized, I_{HTK} showed a decrease in current amplitude compared to neurons that were held at -60mV while currents in hyperpolarized neurons did not change amplitude compared to those clamped at -60mV. In contrast to what was previously reported in IC neurons, I_A does not change in response to any of the stimulations we imposed. When LP neurons were depolarized we found that I_{HTK} also decreased while I_A maintained stable levels whether the neurons were depolarized or maintained at -60mV. This suggests, contrary to our predictions, that K^+ current levels change in response to the same activity pattern. Furthermore, only depolarizing stimulations elicited changes in I_{HTK} while hyperpolarizing and holding at -60mV elicited no change, while IA was not sensitive to stimulation. Although each of these neuron types receives different synaptic input and project to distinct target muscles, the K+ currents of all these cells respond similarly to activity patterns, indicating that there is uniformity in the regulation of intrinsic properties across this motor network. Finally, our data confirm that ionic currents in these neurons may be tuned to specific activity patterns.

Disclosures: D. Salloum: None. **J. Golowasch:** None.

Poster

588. Potassium Channels II

Location: Halls B-H

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Program#/Poster#: 588.03/F10

Topic: B.04. Ion Channels

Support: Telethon Grant GGP11188A

Title: Novel point mutation in the KCNJ10 gene of a severely-disabled patient leads to impaired function of the inwardly-rectifying K^+ channel Kir4.1 and Kir4.1/Kir5.1

Authors: *S. M. HASAN[1,2], A. BALOBAID[3], O. DABBAGH[3], R. RAWASHDA[3], M. PESSIA[1], M. AL-OWAIN[4], M. C. D'ADAMO[1];
[1]Dept. of Exptl. Med., Univ. of Perugia, Perugia, Italy; [2]Dept. of Physiol., Col. of Medicine, Kuwait Univ., Safat, Kuwait; [3]King Faisal Specialist Hosp. and Res. Ctr., Riyadh, Saudi Arabia; [4]Alfaisal Univ., Riyadh, Saudi Arabia

Abstract: A 2 year old boy from non-consanguineous parents presents with tonic-clonic seizures, ataxia, hypotonia, hypothyroidism, profound developmental delay, gut hypomotility, and failure to thrive. EEG recordings were typical of hypsarrhythmia. All family members were clinically unaffected. Genetic screening revealed a novel heterozygous missense variant in the gene KCNJ10 that encodes for the Kir4.1 channel known to be essential for glial function, control of neuronal excitability, and systemic K + homeostasis. The mutation c.652C>T resulted in a leucine to phenylalanine substitution at site 218, a highly conserved residue site located at the c-terminal domain of the channel. To examine the functional consequence of the mutation on Kir4.1, mutant and wild-type KCNJ10 constructs were cloned and heterologously expressed in Xenopus laevis oocytes. Whole-cell K + currents were measured using the two-electrode voltage-clamp technique. Wild-type KCNJ10 expression resulted in robust and typical inward rectifier currents. In contrast, currents from oocytes expressing the mutation were significantly reduced. Kir5.1 subunits display highly selective heteromultimerization with Kir4.1 subunits constituting channels with unique current kinetics. The effect of the mutation on the current from the heteromeric Kir4.1/5.1 channel was examined and was also found to be significantly reduced. In this study, we present a heterozygous KCNJ10 mutation that results in the reduction of inwardly rectifying currents from homomeric Kir4.1 and heteromeric kir4.1/5.1 channels. This loss-of-function mutation was found in a patient with a novel severely-disabling phenotype involving dysfunction of multiple organs.

Disclosures: **S.M. Hasan:** None. **A. Balobaid:** None. **O. Dabbagh:** None. **R. Rawashda:** None. **M. Pessia:** None. **M. Al-Owain:** None. **M.C. D'adamo:** None.

Poster

588. Potassium Channels II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 588.04/F11

Topic: B.04. Ion Channels

Title: Computational studies on Parkinson disease- Role of K-ATP channel in modulating subthalamic nucleus electrical activity

Authors: *C. MAHAPATRA[1], R. MANCHANDA[2];
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Abstract: Increased burst firing in the subthalamic nucleus (STN) has been shown to correlate with symptoms of Parkinson's disease (PD). In STN neurons, intrinsic membrane properties enable burst firing whereas synaptic inputs control the timing of burst discharges. From recent experimental studies, it is documented that ATP-sensitive K+ (K-ATP) current plays as a modulating factor in triggering burst firing. The major key for physiological modeling is a better understanding of the main physiological processes involved. The aim is to establish a mathematical platform of sufficient biophysical detail to quantitatively simulate K-ATP channel in STN neuron model and to investigate contribution of this active conductance in pathophysiological condition of these neuron with respective to PD. The magnitudes and kinetics of this ionic current are described by differential equations, in terms of maximal conductances, electro chemical gradients and ATP dependent activation gating variables. In our model, K-ATP current has reduced the duration of plateau potentials (from 700 ms to 185ms), resting membrane potential (by 10mV) and suppressed NMDA-induced burst firing. Because burst firing in STN neurons is associated with symptoms of Parkinson's disease suppression of bursting may be a potential strategy for improving symptoms of this disease. In summary, this mathematical model provides an elemental tool to investigate the physiological K-ATP channel ionic mechanisms underlying the bursting in STN neuron, which in turn can suggest application of K-ATP channel blocker as a possible treatment for Parkinson disease.

Disclosures: C. Mahapatra: None. **R. Manchanda:** None.

Poster

588. Potassium Channels II

Location: Halls B-H

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Program#/Poster#: 588.05/F12

Topic: B.04. Ion Channels

Support: Fragile X grant DC01919

Autifony Therapeutics

Title: Modulators of Kv3 channels regulate firing rate and temporal accuracy of auditory brainstem neurons in a mouse model of Fragile X syndrome

Authors: *L. EL-HASSAR[1], L. SONG[2], G. ALVARO[3], C. H. LARGE[3], L. K. KACZMAREK[1];
[1]Pharmacol. department SHMB 309, Yale Univ. Sch. of Med., New Haven, CT; [2]Dept. of Surgery, Yale Sch. of Med., New Haven, CT; [3]Autifony Therapeut. Limited, Imperial Col. Incubator, London, United Kingdom

Abstract: Fragile X syndrome (FXS) is the most common form of inherited intellectual disability. In common with autism, FXS is characterized by hypersensitivity to many types of sensory stimuli, including environmental sounds. Our previous work has shown that mice lacking the gene for FMRP (*Fmr1*$^{-/y}$ or Fragile X mice) have abnormally elevated levels of Kv3.1 potassium currents ("high threshold" K^+ currents) and significantly decreased levels of Na^+-activated K^+ currents in auditory brainstem neurons in the medial nucleus of the trapezoid body (MNTB). Both of these changes in K^+ currents are predicted to increase the firing rate of the postsynaptic neurons and to substantially degrade the accuracy of timing of action potentials. Consistent with this, we have found that the firing pattern of MNTB neurons in response to stimulation is severely abnormal in Fragile X mice. The threshold for action potential generation is significantly reduced in Fragile X mice over that in wild type mice. Moreover, in contrast to MNTB neurons from wild type animals, sustained depolarization triggers repetitive firing rather a single action potential at on onset of a stimulus pulse. We have also found that wave IV of the Auditory Brainstem Response (ABR) recorded *in vivo* is significantly enhanced in Fragile X mice, suggesting that loss of FMRP alters central processing of auditory signals. Based on these results we are now testing, in Fragile X mice, the physiological effects of potential therapeutic compounds, AUT2, which modulate the activity of Kv3 family channels in cell lines. We found that, in Fragile X mice, AUT2 improved the accuracy of timing of action potentials in response to repetitive stimulation, presumably by shifting the activation curve of high threshold potassium currents to hyperpolarizing potentials, thereby increasing the low threshold potassium currents and restoring the accuracy and the timing of action potentials. We are now testing the effects of the AUT2 compound on ABR *in vivo*.

Disclosures: L. El-Hassar: None. **L. Song:** None. **G. Alvaro:** None. **C.H. Large:** None. **L.K. Kaczmarek:** None.

Poster

588. Potassium Channels II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 588.06/F13

Topic: B.04. Ion Channels

Support: CIHR(RWT)

Title: The ERK signaling pathway as a component of the Cav3-Kv4 interaction

Authors: *X. ZHAN, A. P. RIZWAN, H. ASMARA, R. W. TURNER;
Cell biology and Anat., Univ. of Calgary, Calgary, AB, Canada

Abstract: Our previous work revealed an interaction between Cav3 T-type calcium channels and Kv4 A-type potassium channels that can regulate A-type (IA) current availability in cerebellar stellate and granule cells. Cav3-mediated calcium influx increases I_A by rightward shifting the half-inactivation potential (Vh) of Kv4 channels through a nanodomain interaction supported by an association between Cav3 and Kv4 C-termini. We know that the Cav3-Kv4 interaction depends on the calcium-sensitive potassium channel interacting protein isoform 3 (KChIP3), but the molecular basis by which calcium signals a KChIP3-mediated change in Kv4 Vh has yet to be determined. In order to define the process for calcium-dependent activation of KChIP3 and the Cav3-Kv4 interaction, we conducted coimmunoprecipitations (co-IPs) from tsA-201 cells and found a calcium-independent coIP between Kv4 and KChIP3. A co-IP was also found between Cav3.1 and KChiP3 that was reduced in nominally free calcium (BAPTA-AM) but consistent for physiological levels of calcium. Whole-cell voltage-clamp recordings were conducted in tsA-201 cells transfected with Cav3.1, Kv4.3, and KChIP3 cDNA, or a KChIP3 mutant construct lacking two calcium binding EF hand motifs (E186Q, E234Q). Applying mibefradil or Ni^{2+} to block Cav3 channels produced a leftward shift of I_A Vh in cells expressing wildtype KChIP3, but not in cells expressing the KChIP3 double mutant. The mibefradil-induced shift in IA Vh was also blocked by applying a tat peptide (TAT-PP1) against amino acids 7-23 of the Kv4 N-terminus involved in binding KChIP3 to Kv4.3. Moreover, intracellular application of PD98059 to block phosphorylation of MEK but not AIP to block CaMKII inhibited the mibefradil-induced shift in IA Vh. The results indicate that calcium regulation of Kv4 availabilty depends on the the integrity of the KChIP3 - Kv4 interaction site and involves both the ERK signaling pathway and EF hand calcium-binding motifs of KChIP3.

Disclosures: X. Zhan: None. **A.P. Rizwan:** None. **H. Asmara:** None. **R.W. Turner:** None.

Poster

588. Potassium Channels II

Location: Halls B-H

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Program#/Poster#: 588.07/F14

Topic: B.04. Ion Channels

Support: NIH Grant R01AG022508

NIH Grant R01EY021796

Title: A glial K^+ channel augments *Drosophila* visual contrast sensitivity by facilitating inhibitory synaptic transmission

Authors: Z. LUAN, 01605, P. GUO, *H.-S. LI;
Neurobio., Univ. Mass. Med. Sch., Worcester, MA

Abstract: The mechanism regulating visual neuronal circuits by glial cells to attain contrast sensitivity in human and animal eyes is unknown. Here we report an essential role for a glial inwardly rectifying K^+ channel (Kir) Irk2 in *Drosophila* visual contrast sensitivity. Both systemic and glia-specific Irk2 deficiencies caused electrical oscillation in electroretinogram during light stimulation, and reduced fly behavioral responses to moving gratings at low contrast. Further analyses indicated that Irk2 in glia controls light-triggered extracellular K^+ increase in the visual ganglion lamina and prevents abnormal excitation of laminar neurons postsynaptic to histaminergic photoreceptors, suggesting the importance of glia-mediated K^+ buffering to inhibitory synaptic transmission. Since decreased glial Kir channel activities are observed in human diabetic retinopathies with reduced contrast sensitivity, this work may have revealed a conserved glial function that optimizes visual contrast sensitivity.

Disclosures: **Z. Luan:** None. **P. Guo:** None. **H. Li:** None.

Poster

588. Potassium Channels II

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Program#/Poster#: 588.08/F15

Topic: B.04. Ion Channels

Support: NIH Grant R01NS062720

NMSS Grant TA3012A1

Title: Radial glial activation by Kv3 deletion in suppressing inflammatory demyelinaton and axon degeneration

Authors: *C. GU;
Biol. Chem. and Pharmacol., Ohio State Univ., Columbus, OH

Abstract: The development of neuroprotective and repair strategies for treating progressive multiple sclerosis (MS) requires new insights in axonal injury and recovery. 4-aminopyridine (4-AP), a blocker of voltage-gated K^+ (Kv) channels, is used in symptomatic treatment of progressive MS, but the underlying mechanism remains unclear. Here we report that deleting Kv3—a channel with the highest 4-AP sensitivity—reduces clinical signs in experimental autoimmune encephalomyelitis (EAE), a mouse model for MS. In Kv3 knockout (KO) mice, EAE lesions in both sensory and motor tracts of spinal cord markedly reduced, and radial astroglia were activated with increased expression of brain derived neurotrophic factor (BDNF). Kv3 and activated BDNF receptors were upregulated in demyelinating axons in EAE and MS lesions. In spinal cord myelin coculture, BDNF enhanced myelination and altered neuronal firing partially through upregulating Kv3. Therefore, suppressing Kv3 increases neural activity and enhances BNDF signaling, which may protect axons from inflammatory injury.

Disclosures: C. Gu: None.

Poster

588. Potassium Channels II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 588.09/F16

Topic: B.04. Ion Channels

Title: Calcium activated potassium currents in different cell types of the neonatal hippocampus

Authors: A. MONICAL, *M. MYNLIEFF;
Biol. Sci., Marquette Univ., Milwaukee, WI

Abstract: Calcium (Ca^{2+}) regulates many different processes in neurons, including excitability. One way in which Ca^{2+} influx regulates excitability is by activation of Ca^{2+} activated potassium (K^+) channels such as BK, SK and KCNQ channels, which may be involved in mediating action potential repolarization and afterhyperpolarization. The expression of K^+ channels is not uniform across cell types so in the wide variety of neurons in the hippocampus (up to 40 types), a different complement of K^+ channels may contribute to excitability. The various interneuron subtypes play different roles in controlling excitability and overall output of the pyramidal cells. Understanding how Ca^{2+} activated K^+ channels control the excitability of the different cell types will contribute to our appreciation of how modulation of Ca^+ influx can regulate excitability in single cell types as well as excitability of the overall circuitry. In this study whole cell patch clamp recording was used to measure K^+ currents in primary cultures of the superior region of neonatal rat hippocampus. The total K^+ current demonstrated a fast activing, transient current (I_A) and long lasting, sustained current (I_K). Currents were elicited by stepping from -50 to +50

mV in 10 mV increments from -90 mV for total current or -50 mV for I_K. I_A was determined by subtracting I_K from the total current. The cells were divided into three groups based on the rise time of the total current: cells with a slow rise time and no I_A, cells with an intermediate rise time and I_A, and cells with a fast rise time and large I_A. This classification also corresponded to cell size, with smaller cells having less I_A. Nimodipine (40μM), an L-type Ca^{2+} channel antagonist, blocked 35.5% of I_K in cells with little I_A (N=2), blocked 81.3±4.65% of I_K in cells with intermediate I_A (N=3), and 65.3±5.74% of I_K in cells with large I_A (N=11). These data suggest that Ca^{2+} influx through L-type channels activates K^+ channels involved in I_K. In cells with a large I_A (N=11), nimodipine blocked 29.9±4.55% of the I_A current suggesting that influx of Ca^{2+} through L-type channels contributes more to I_K than to I_A . Two large conductance (BK) Ca^{2+} activated K^+ channels antagonists, iberiotoxin (10 μM; N=7) and verruculogen (10 nm; N=4) had very little effect on the total I_A and I_K in all of the three cell types. The effect of nimodipine and selective K^+ channel antagonists on action potential and afterhyperpolariztion duration will be tested in heterogeneous neonatal cells. Co-localization of specific Ca^{2+} activated K^+ channels and various interneuron markers will be used to correlate the electrophysiological findings with interneuron identities.

Disclosures: A. Monical: None. **M. Mynlieff:** None.

Poster

588. Potassium Channels II

Location: Halls B-H

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Program#/Poster#: 588.10/F17

Topic: B.04. Ion Channels

Support: UC Davis startup

NIH CounterACT Program (U54NS079202)

Title: Structural insights into the mechanism of KCa3.1 channel-small molecule interactions

Authors: *H. M. NGUYEN[1], V. SINGH[1], H. WULFF[1], V. YAROV-YAROVOY[2];
[1]Pharmacol., [2]Physiol. and Membrane Biophysics, UC Davis, Davis, CA

Abstract: Ion channels play a key regulatory role in many biological processes and thus are a major pharmacological target for drug development. The calcium-activated intermediate-conductance KCa3.1 channel is an effective regulator of intracellular calcium and an attractive pharmacological target for immunosuppression, fibroproliferative disorders, hypertension and various neurological diseases. However, the development of drugs for this medically relevant

channel is hindered by the unavailability of a crystal structure useful for structure-guided drug design. To gain insights into the possible active sites underlying channel-small molecule interactions, we utilized the Rosetta molecular modeling suite to generate a homology model of the KCa3.1 channel transmembrane region using the Kv1.2-Kv2.1 channel structure (pdb id: 2R9R) as a template. Upon docking of known KCa3.1 small molecule blockers into this homology KCa3.1 model, we identified, surprisingly, two independent sites for the dihydropyridine nifedipine and for its isoster methyl-5-acetyl-4-(4-chloro-3-(trifluoromethyl)phenyl)-2,6-dimethyl-4*H*-pyran-3-carboxylate. While nifedipine is predicted to bind in the fenestration between the pore-lining S5 and S6 segments, the pyran isoster has its lowest energy binding configurations in the inner pore region. We then tested and confirmed these predictions via a combination of site-directed mutagenesis and electrophysiological patch-clamp recording. Blocking of KCa3.1 by nifedipine was significantly reduced by replacing the side chains at the fenestration positions L209, T212 and V272 with either alanine or valine. Replacement with bulkier phenylalanines confirmed T212 and V272 as the main interacting sites for nifedipine without compromising the affinity of inner pore blockers like TRAM-34 or the 4-phenyl pyran. In contrast, the inner pore T250S and V275A mutants, which are known to nullify TRAM-34 binding disrupted binding of the 4-phenyl pyran but did not interfere with nifedipine binding. We conclude that the Rosetta modeling approach can be useful to distinguish the molecular mechanisms of action of KCa channel modulators and has promising potential in guiding the development of clinically relevant drugs.

Disclosures: H.M. Nguyen: None. **V. Singh:** None. **H. Wulff:** None. **V. Yarov-Yarovoy:** None.

Poster

588. Potassium Channels II

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Topic: B.04. Ion Channels

Support: DFFD Grant F46.2/001

NASU Biotechnology

NASU Functional Genomics and Metabolomics Grant

NASU Grant 67/15-H

Title: Signaling of neuronal Ca^{2+} sensor proteins in hippocampal neurons

Authors: N. I. KONONENKO[1], J. VIVIANO[2], A. V. DOVGAN[1], V. P. CHERKAS[1], J. ZHANG[2], V. VENKATARAMAN[2], *P. V. BELAN[1,3];
[1]Bogomoletz Inst. of Physiol., Kiev, Ukraine; [2]Dept. of Cell Biology, Grad. Sch. of Biomed. Sci., Rowan Univ. Sch. of Osteo. Med., Stratford, NJ; [3]Intl. Ctr. for Mol. Physiol., Kiev, Ukraine

Abstract: Structurally similar neuronal Ca^{2+} sensor (NCS) proteins, Neurocalcin δ (NCALD) and Hippocalcin (HPCA), control many neuronal processes including slow afterhyperpolarization (sAHP) and long-term depression (LTD). Both sAHP, which tunes neuronal activity, and LTD, which regulates the synaptic strength, are likely controlled by HPCA and NCALD translocation from the cytosol to the plasma membrane. Difference in AA sequence between HPCA and NCALD suggests different Ca^{2+}-dependent signaling of these proteins. To test this hypothesis we studied $[Ca^{2+}]_i$ regulation and Ca^{2+}-dependent translocation of HPCA, NCALD, and their chimeras and mutants tagged by different fluorescent proteins. Minor increases in $[Ca^{2+}]_i$ were observed when the resting membrane potential was increased stepwise in a range of from -80 through -40 mV. These increases resulted in gradual translocation of NCALD rather than HPCA to the plasma membrane thus activating membranous NCALD targets and depriving a cytosolic pool of NCALD for the following fast translocation. The fast depolarization-induced $[Ca^{2+}]_i$ transients led to different time courses, amplitudes, and locations of fast translocation of HPCA, NCALD and their chimeras and mutants. We found that N-terminal AA sequence including EF1 domain was important determinant for differences in kinetics, localization and Ca^{2+}-dependency of NCS protein signaling. NCALD mutants, in which dimerization was prevented, did not reveal substantial translocation indicating that Ca^{2+}-dependent dimerization may be necessary for NCALD translocation. We conclude that structural distinctions in AA sequence of HPCA and NCALD may result in differential Ca^{2+}-dependent regulation of their targets and plasma membrane conductance, thus far differentially regulating dendritic integration and synaptic plasticity.

Disclosures: N.I. Kononenko: None. **J. Viviano:** None. **A.V. Dovgan:** None. **V.P. Cherkas:** None. **J. Zhang:** None. **V. Venkataraman:** None. **P.V. Belan:** None.

Poster

588. Potassium Channels II

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Program#/Poster#: 588.12/F19

Topic: B.04. Ion Channels

Support: Department of Health and Human Services/ National Institutes of Health/ National Institute on Drug Abuse/ Intramural Research Program

Title: A single pre-exposure to methamphetamine potentiates methamphetamine self-administration and attenuates changes in DNA methylation and expression of potassium channels in the rat nucleus accumbens.

Authors: *S. JAYANTHI, M. GERRA, N. TERRY, M. MASNATA, B. LADENHEIM, I. N. KRASNOVA, M. T. MCCOY, J. L. CADET;
Mol. Neuropsychiatry Br., NIDA-IRP, Baltimore, MD

Abstract: Drug addiction is a chronic relapsing neuropsychiatric disorder associated with transcriptional and epigenetic changes in brain reward circuitries. Methamphetamine (METH) addiction is a major problem in the world with its prevalence outpacing that of cocaine. Transition from occasional use to dependence state can be accelerated by un-prescribed use of psychostimulants. An earlier study from our lab has shown that a single non-contingent METH injection increased cocaine self-administration (SA). Although the molecular mechanisms underlying this behavioral response to cocaine is not known, a single METH injection has also been shown to impact gene expression and DNA methylation in the brain. In the present study, we tested the possibility that a single METH injection could impact METH SA. Towards that end, we administered a single dose of METH (10 mg/kg) or saline to rats prior to training them to self-administer METH (0.1mg/kg, 3h-6h/day, 18days). This approach resulted in three experimental groups: (1) control- saline-saline group (SS), (2) single saline injection followed by METH SA (SM) and (3) single METH injection followed by METH SA (MM). We found that the MM group showed significantly higher METH intake than the SM group. Cue-induced METH seeking was examined using extinction tests on day 2 (WD2) and 29 (WD29) of forced withdrawal from METH SA. Both SM and MM groups showed higher cue-induced reward seeking on WD29 compared to WD2. There were no differences between the SM and MM groups. Because our recent genome-wide DNA hydroxymethylation experiment had identified potassium channels in the nucleus accumbens as discriminators of compulsive METH takers and abstinent rats, we wondered if changes in the expression of potassium channels might help us to distinguish SM from MM groups. Interestingly, the SM rats showed increased mRNA expression of *shaker-related* voltage-gated potassium channels (Kv1-*Kv1.1, Kv1.3, and Kv1.6*) in comparison to MM rats. SM rats also showed decreased DNA methylation at the CpG-rich sites near the promoter region of *Kv1.1* and *Kv1.3* genes in comparison to MM rats. Together, our results provide further evidence for the potential involvement of potassium channels in METH addiction.

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Poster

589. Postsynaptic Organization and Structure II

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Program#/Poster#: 589.01/F20

Topic: B.07. Synaptic Transmission

Support: NIH Grant 5R01DA039533-02 to F.S.N

Title: Epigenetic reversal of maternal deprivation-induced dopamine cell hyperexcitability and synaptic dysfunction within the VTA

Authors: *H. KASSIS, L. D. LANGLOIS, S. GOUTY, M. E. AUTHEMENT, R. D. SHEPARD, B. M. COX, F. S. NUGENT;
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Abstract: Child abuse and neglect as early life stressors are shown to increase the risk of developing stress-related disorders and substance abuse. The increased vulnerability seems to be related to brain monoaminergic dysfunction including an altered dopamine (DA) signaling from the ventral tegmental area (VTA). Recently, we demonstrated that a 24h early maternal deprivation (MD, an animal model of child abuse), on postnatal day 9, induces synaptic abnormalities at GABAergic synapses onto VTA DA neurons through disruption of A kinase anchoring protein (AKAP150) signaling in juvenile rats (p14-p21). These synaptic defects were normalized by *in vitro* histone deacetylase (HDAC) inhibition suggesting the potential clinical benefits of targeting the VTA by HDAC inhibitors soon after the insult. Here we further investigated the synaptic and epigenetic modifications associated with MD in VTA DA neurons and tested the effects of a single in vivo injection of a selective class I HDAC inhibitor (CI-994) on these MD-induced changes within the VTA. We found that MD indeed increased HDAC2 (a class I HDAC) expression specifically in VTA DA neurons resulting in reduction of histone H3 acetylation at lysine 9 (Ac-H3K9). The levels of Ac-H3K9 in VTA of MD rats were restored 3 hours after a single *in vivo* injection of MD animals with CI-994. We also detected that MD induced DA cell hyperexcitability which was also normalized by the *in vivo* HDAC inhibition. Our preliminary data suggest that GABAergic and glutamatergic synaptic dysfunction could also be reversed through restoration of synaptic localization of AKAP shortly after a single *in vivo* injection of CI-994. Taken together, our results suggest that a single *in vivo* HDAC inhibition may be sufficient to epigenetically reverse MD-induced changes in AKAP localization, DA cell excitability and synaptic dysfunction within the VTA.

Disclosures: H. Kassis: None. **L.D. Langlois:** None. **S. Gouty:** None. **M.E. Authement:** None. **R.D. Shepard:** None. **B.M. Cox:** None. **F.S. Nugent:** None.

Poster

589. Postsynaptic Organization and Structure II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

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Topic: B.07. Synaptic Transmission

Support: SFRH/BD/51960/2012

PTDC/NEU-NMC/4888/2014

Title: Role of ARHGAP8, a novel RhoGAP, in regulating excitatory synapses

Authors: J. SCHMIDT[1,2,4], J. FERREIRA[6], *C. B. DUARTE[7,3], A. CARVALHO[1,5];
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Abstract: Normal brain function is strongly dependent on the correct development and assembly of the neuronal cytoskeleton. Failure to achieve the correct neuronal layout or to form the right interconnections between nerve cells underlies disorders involving cognitive deficits and mental disability. During development and other processes that include changes in cell architecture, as seen for example in plasticity, neurons have to undergo in some cases extensive reorganization of their actin scaffolding. Synaptic plasticity is associated with mechanisms such as the dynamic changes in spine size and number and the ability of excitatory glutamate-activated synapses to alter their strength in response to changes in activity patterns. Members of the Rho small GTPase subfamily have been shown to orchestrate many cellular processes involving intraneuronal actin dynamics. Essentially working like binary switches, they cycle between inactive GDP-bound states and active GTP-bound states, to which they are driven by GTPase activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs), respectively. A variety of these regulators have by now been shown to play critical roles within neurons. We identified ARHGAP8 as a novel Rho GAP which is localized to spines in a GluN2B-NMDA receptor dependent manner. Using quantitative mass spectrometry, we have found ARHGAP8 to be absent from postsynaptic densities (PSDs) of mouse neurons that lack the developmentally regulated NMDA-type glutamate receptor subunit GluN2B, while it is identified in PSDs isolated from wild-type neurons. We are currently characterizing its function within neurons and our data from biochemical and immunocytochemical experiments suggest a brainwide expression pattern and a partial localization to active synapses. Furthermore, ARHGAP8

overexpression in rat neuronal cultures affects spine maturation. Overall our data suggest that ARHGAP is a novel regulator of excitatory synapses.

Disclosures: **J. Schmidt:** None. **J. Ferreira:** None. **C.B. Duarte:** None. **A. Carvalho:** None.

Poster

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Topic: B.07. Synaptic Transmission

Support: NINDS & NIBIB intramural funds

NIH grant R01NS040701

Title: Distinct molecular conformations of AKAP79/150 in hippocampal spine synapses

Authors: ***X. CHEN**[1], A. FENG[2], K. CROSBY[3], A. PURKEY[3], C. WINTERS[2], V. CROCKER[2], M. ARONOVA[4], R. LEAPMAN[4], T. REESE[2], M. DELL'ACQUA[3]; [1]Lab. Neurobiol, [2]NINDS-NIH, Bethesda, MD; [3]Dept. of Pharmacol., Univ. of Colorado Sch. of Med., Aurora, CO; [4]NIBIB-NIH, Bethesda, MD

Abstract: Protein kinase A (PKA), protein kinase C (PKC) and calcineurin (CaN) are signaling molecules working in conjunction with AMPA-type and NMDA-type glutamate receptors in long-term potentiation (LTP) and depression (LTD). While glutamate receptors are localized to the synaptic membrane through the PSD-95 family of MAGUK scaffold proteins, these kinases and phosphatases are targeted to the synapses through binding to the scaffold protein A-Kinase anchoring protein (AKAP) 79/150. AKAP79/150 contains a membrane-targeting domain characterized by three subdomains with a PKC anchoring site at the N-terminus, a MAGUK binding region downstream, and CaN and PKA anchoring sites at the C-terminus. Despite extensive studies of the function of this AKAP, very little is known about its nanometer scale localization and molecular conformation at the spine membrane and at the postsynaptic density (PSD). We expressed N or C terminally GFP tagged AKAP79 constructs in rat hippocampal cultures and used immunogold EM to map their localization and determine their conformations at the spine membrane and in the PSD. We also used scanning transmission electron microscopy (STEM) tomography to reconstruct entire PSDs at 6 nm resolution in spine membranes positively labeled for AKAP79. Our results show extensive AKAP localization at the spine membrane both outside the PSD and in regions within the PSD. STORM/PALM super-resolution fluorescence microscopy with mEOS-tagged AKAP79 confirmed distinct clustered localizations

in spines both outside the PSD and overlapping with PSD-95 in the PSD. At the spine membrane outside the PSD, EM also revealed that the N and C termini of AKAP79 are located at similar distances from the plasma membrane, suggesting they assume a closed or horizontally extended orientation. At the PSD, the N terminus is located near the membrane but the C terminus is located ~ 10 nm away, suggesting that AKAP79/150 molecule is vertically oriented relative to the membrane and in an extended conformation. These results demonstrate that AKAP79 adopts distinct molecular conformations at the PSD different from those at the spine membrane outside the PSD. Our results provide an initial look at the molecular organization of the AKAP79/150 postsynaptic signaling complex in dendritic spines and might lead to further understanding of the molecular inner-workings of the PSD in synaptic transmission and plasticity.

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Poster

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Title: Lithium increases synaptic GluA2 by elevating the autism-related δ-catenin protein in hippocampal neurons

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Abstract: Lithium (Li+) is a drug widely employed for treating bipolar disorder, however the mechanism of action is not known. Here we study the effects of Li+ in cultured hippocampal neurons on a synaptic complex consisting of δ-catenin, a protein associated with cadherins whose mutation is linked to autism, and GRIP, an AMPA receptor (AMPAR) scaffolding protein, and the AMPAR subunit, GluA2. We show that Li+ elevates the level of δ-catenin in cultured neurons. This elevation is consistent with Li+ inhibition of GSK3β, a kinase that phosphorylates and destabilizes δ-catenin. δ-catenin binds to the ABP and GRIP proteins, which are synaptic scaffolds for GluA2. We show that Li+ increases the levels of GRIP and GluA2, consistent with Li+-induced elevation of δ-catenin. Using GluA2 mutants, we show that the

increase in surface level of GluA2 requires GluA2 interaction with GRIP. Furthermore, Li+ fed animals show elevated synaptic levels of δ-catenin, GRIP, and GluA2 in the hippocampus, consistent with the finding in cultured neurons. This work supports a model in which Li+ stabilizes δ-catenin by inhibition of GSK3β, elevating δ-catenin, GRIP and GluA2 in synapses of hippocampal neurons. Thus, it suggests a mechanism by which Li+ can alter brain synaptic function that may be relevant to its pharmacologic action in treatment of neurological disease.

Disclosures: S. Kim: None. **M. Farooq:** None. **S. Patel:** None. **L. Khatri:** None. **E. Ziff:** None.

Poster

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GM102575

DA022727

MH086425

MH100093

Title: Phosphorylation of EphB2 receptor tyrosine kinase ectodomain

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Abstract: Extracellular phosphorylation of proteins was suggested in the late 1800's when casein was shown to contain phosphate. More recently extracellular kinases that phosphorylate

extracellular serine, threonine, and tyrosine residues of numerous of proteins have begun to be identified. However, the significance of extracellular phosphorylation of specific residues for protein function in the nervous system is poorly understood. Crucial for proper synaptic development is the EphB family of receptor tyrosine kinases and their membrane bound ligands, the ephrin-Bs. The binding of ephrin-B2 to the ligand binding domain (LBD) of EphB2 drives a direct extracellular interaction between the EphB2 receptor and the N-methyl-D-aspartate receptor (NMDAR), an ionotropic glutamate receptor required for synaptic plasticity. Normal brain function requires that NMDARs are properly localized to synaptic sites, whereas absence of the NMDAR is lethal in mice. EphB2 is necessary for the correct localization and function of NMDAR at synapses. However, because the interacting domains between EphB2 and the NMDAR are yet to be determined, the mechanisms enabling extracellular interactions have not been well studied. We demonstrate that EphB2 undergoes post-translational modification of its extracellular domain that enables it to interact with the NMDAR. Unbiased mass spectrometry data suggest that a specific tyrosine reside, Y504, in the fibronectin type III (FN3) repeat domain of the extracellular region of EphB2 undergoes ephrin-B2 ligand-dependent phosphorylation. Furthermore, mutation of the predicted extracellular phosphorylation site shows it is required for the EphB-NMDAR interaction. Y504 is phosphorylated in both the brain and spinal cord and data show that constitutive phosphorylation of this site results in mechanical hypersensitivity in mice expressing a phospho-mimetic mutant of EphB2. Given that several recent publications have demonstrated the existence of ectokinases and ectophosphatases, we are investigating the possibility that a secreted kinase is a candidate for extracellular phosphorylation of EphB2. Because of the importance of the Eph-NMDAR interaction, identifying the extracellular kinase allows for a potential therapeutic target as well as insight into the phenomenon of extracellular phosphorylation. Taken together, our results suggest that extracellular phosphorylation by a secreted kinase might be an underappreciated mechanism in regulation of protein interaction and protein trafficking.

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Poster

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Howard Hughes Medical Institute

Grass Foundation

Title: NMDA receptor mediated activation of pannexin1 channels at hippocampal mossy fiber synapses support burst-firing output in CA3 pyramidal neurons

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Abstract: Pannexin1 channels are a distinct category of large pore ion channels expressed throughout the mammalian brain. While pannexins have been shown to participate in various neuropathologies, the extent of their physiological role remains largely unexplored. Here we combine pharmacological and genetic tools with acute slice electrophysiology to demonstrate NMDA receptor (NMDAR)-mediated activation of pannexin1 channels in CA3 pyramidal neurons at hippocampal mossy fiber synapses. Pannexin1-mediated secondary currents are revealed at physiological temperature (35 °C) but not at room temperature (25 °C), and probabilistically occur following the peak of NMDAR-mediated synaptic responses. These currents are abolished by the pannexin1 inhibitory peptide Panx10 (50 μM), carbenoxolone (30 μM), and intracellular loading of BAPTA (10 mM). Additionally, we provide immunohistological evidence that pannexin1 is highly expressed in *stratum lucidum,* and immunoelectron microsocopy revealed pannexin1 near the postsynaptic density of thorny excrescences. Moreover, we show that pannexin1 channels are preferentially recruited when NMDAR activation enters a supralinear regime, resulting in burst-firing output. In line with the hypothesized role for mossy fiber synapses as potent drivers or "detonators" of CA3 pyramids during episodic memory, we found that by mimicking the structure of *in-vivo* activity, pannexin1 mediated secondary currents can be bidirectionally modulated by burst-timing dependent plasticity of NMDAR-mediated transmission. Our data suggest that pannexin1-mediated secondary currents provide a mechanism for signal amplification, eliciting temporally delayed burst-firing of hippocampal CA3 pyramidal neurons. This delay likely enables particularly salient stimuli that drive NMDARs into a supralinear regime, to extend associativity windows of co-activated CA3 pyramids during auto-associative functions of the CA3 region of the hippocampus.

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Poster

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Support: CCTSI Center for Neuroscience Pilot Award

Title: Investigating the role of csmd2 in reelin-dab1 function at synapses

Authors: B. E. DWYER, M. A. GUTIERREZ, *S. J. FRANCO;
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Abstract: Reelin is a secreted glycoprotein that regulates the development and function of the cerebral cortex by initiating a signaling cascade via its downstream adaptor protein Dab1. The developmental roles of Reelin/Dab1 signaling have been extensively studied, and many of the molecular and cellular mechanisms of Reelin's critical functions during neuronal migration and differentiation have been elucidated. On the other hand, the role and mechanisms of Reelin signaling in the mature brain are considerably less understood. Reelin continues to be secreted by inhibitory interneurons in the adult forebrain and emerging evidence suggests Reelin signaling is important for synapse function and learning and memory. However, the mechanisms that control synapse function and brain physiology downstream of Reelin and Dab1 are not known. We have identified a novel interaction between Dab1 and Csmd2, which belongs to a family of single-pass transmembrane proteins of unknown function. We found that the cytoplasmic tail of Csmd2 binds to Dab1 via an NPxY motif, and to several synaptic scaffolding proteins via a PDZ-binding domain. Csmd2 localizes to synapses in vitro in rat primary hippocampal cultures, as well as in vivo in mouse cerebral cortex. Introduction of a truncated form of Csmd2 into mouse cortical neurons by in utero electroporation caused a dramatic reduction of dendritic spine number and density in vivo. This phenotype was relieved in a mutated form of the truncated protein that no longer localizes to synapses. Together these data indicate a role for Csmd2 in synapse formation, maintenance and/or function. Based on the similarities between the extracellular domain architecture of Csmd2 and several synaptic auxiliary proteins, we are now testing the hypothesis that Csmd2 serves as an auxiliary subunit of ionotropic glutamate receptors. We have also begun testing the hypothesis that the interaction between Dab1 and Csmd2 is required for proper synapse function, and that Reelin signaling modulates this interaction.

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Poster

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Support: UID/NEU/04539/2013

PTDC/NEU-NMC/0750/2012

SFRH/BD/51962/2012

Title: CACNG2, a new candidate for schizophrenia and intellectual disability

Authors: G. L. CALDEIRA[1], S. R. LOUROS[2], M. V. RODRIGUES[3], C. N. PATO[4], C. CHINFEI[5], J. PEÇA[6], *A. L. CARVALHO[7];
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Abstract: Schizophrenia is a devastating disorder that affects about 1% of the worldwide population. This multifactorial disease, with a strong genetic component, is characterized by delusions, hallucinations and confusion thoughts. Furthermore schizophrenia onset and stress are inextricably related. It has been suggested that schizophrenia patients present a disruption of normal homeostatic adaptation of neuronal circuits to changes in the environment, as a consequence of impaired synaptic function, since several *de novo* mutations in synaptic networks components have been found in these patients. Stargazin is an auxiliary subunit for AMPAR and it is required for AMPAR trafficking to the surface and for the homeostatic scaling up of AMPAR upon blockade of activity. Using whole-genome sequence analysis, we identified a new CACNG2 variant, Stg^{SCZ}, which, along with Stg^{ID}, previously identified in intellectual disability, altered the surface trafficking properties of stargazin and failed to deliver AMPAR to synapses. Furthermore, Stg^{ID} was not able to mediate homeostatic plasticity whereas Stg^{SCZ} increased the number and decreased the length of primary dendrites, as well as the number of inhibitory synapses. Tianeptine, a commercial memory enhancer and powerful antidepressant, described to increase AMPAR trapping at synapses by increasing stargazin phosphorylation, rescued AMPAR levels in neurons expressing STG^{SCZ}. The correlation between stargazin disease-related

variants and specific alterations in the function of the protein will allow elucidating the mechanisms underlying the development of these neuropsychiatric disorders.

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Poster

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NARSAD Young Investigator

Chicago Biomedical Consortium Catalyst Award

Hartwell Foundation Individual Biomedical Research Award

Title: Analysis of specific synaptic proteomes in the mouse brain

Authors: *J. N. SAVAS, Y.-Z. WANG, C. STROJNY;
Northwestern University, Feinberg Sch. of Medici, Chicago, IL

Abstract: Synapses are protein rich cell junctions and synaptic malfunction plays a key role in many neurodevelopment disorders and neurodegenerative diseases. While a near comprehensive description of the proteins which can be present at synapses has been obtained, an understanding of which specific proteins are present at distinct synapses represents a critical gap in our understanding of synapse diversity. Up till now, there have been only a few reports which have described neuron-specific proteomes and none have investigated neuron-specific perturbations. In traditional neuroproteomic mass spectrometry (MS) studies, samples are prepared from brain which represent complex mixtures of proteins from multiple cell types and suffer from "averaging". If one synapse is affected while another is not, and tissue is simply homogenized, the resulting analysis can be misleading since the measured signal will be an average. To overcome this limitation, we developed a new approach which couples synaptic membrane targeting, proximity biotin ligation, conditional expression, and isotope based quantitative MS analysis.
We fused BirA* biotin ligase with the transmembrane regions of NRXN1 or NLGN1 (from GRASP), which will target pre- or post-synaptic locations. As expected, both BirA*-pre and -

post are expressed on the cell surface. Our constructs exhibited strong biotinylation activity and when expressed in rat hippocampal neurons, both BirA*-pre and -post showed punctate distribution patterns. Based on co-localization with synaptic markers our fusion proteins predominantly localized to excitatory and to a lesser degree inhibitory synapses. Next, we moved our fusion proteins into AAV-FLEx vectors which express only in presence of Cre and injected AAVs into the striatum of dopamine receptor -1 (D1) – and (D2) - Cre mice. Biotin was injected, and the biotinylated proteins were isolated with streptavidin, and MS analysis identified a contrasting set of proteins. By crossing different Cre lines with various mouse models of neurological disease we will monitor specific synaptic proteomes over the time course of disease onset and progression. We believe that determining specific synaptic perturbations on the proteome level will provide new insight into neurological disease.

Disclosures: **J.N. Savas:** None. **Y. Wang:** None. **C. Strojny:** None.

Poster

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NIH intramural PRAT fellowship

Title: Role of phosphorylation of Neuroligin-2 at inhibitory synapses

Authors: ***N. F. SHANKS**[1,2], M. A. BEMBEN[1], Y. LI[1], K. W. ROCHE[1];
[1]NINDS, [2]Nigms, NIH, Bethesda, MD

Abstract: Integration between excitatory and inhibitory neurotransmission is critical for proper brain function, and disruptions of this balance have been implicated in many neurological disorders including epilepsy, schizophrenia, and autism spectrum disorders. Neuroligins (NLs) are postsynaptic cell adhesion molecules that are ubiquitously expressed in the brain, and are thought to play pivotal roles in synapse assembly, organization and function. The NL isoform, NL-2, is located and specifically functions at inhibitory synapses, whereas other NL isoforms function only at excitatory synapses or can act at both synapse types. Phosphorylation of other NL isoforms has been shown to modulate their function at synapses, and we believe that phosphorylation may be a mechanism involved in their differential roles at inhibitory vs. excitatory synapses. We demonstrate that NL-2 is a substrate for c-AMP dependent kinase

(PKA), and using mass spectrometry determined the exact residue in the intracellular C-tail that is phosphorylated. We have generated a phospho-specific antibody against this site, and demonstrate it's specificity. With this great tool, we are now characterizing NL-2 phosphorylation and regulation. We observe that NL-2 is basally phosphorylated in cultured cortical rat neurons and in rodent brain, and we are characterizing NL-2 phosphorylation over developmental time points, in different brain regions, and in different brain fractions. We have demonstrated that NL-2 phosphorylation is regulated by synaptic activity, and are investigating the precise mechanisms by which this occurs. Finally, by comparing the function of NL-2 phospho mutants, we have observed that this NL-2 phosphorylation site is important for inhibitory synapse function.

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Poster

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Support: NINDS

Title: Differences in phosphorylation of neuroligins: 4x vs. 4y

Authors: ***T. A. NGUYEN**[1,2], M. A. BEMBEN[1], Y. LI[1], K. W. ROCHE[1];
[1]NINDS, Bethesda, MD; [2]Pharmacol. and Physiol., Georgetown Univ., Washington, DC

Abstract: Autism Spectrum Disorders (ASDs) are a diverse set of cognitive developmental disorders that result in a wide range of behavioral deficits. Interestingly, ASDs have long been reported to affect many more males than females. This sex bias in ASDs has been a puzzle in the field. Neuroligins (NLs) are postsynaptic cell adhesion molecules involved in synapse formation and modulation. There are five NLs (NL-1, NL-2, NL-3, NL-4X, NL-4Y) encoded in the human genome, whereas in rodent there are four (NL-1, NL-2, NL-3, NL-4-like). NL-4X and NL-4Y are of particular interest because they are sex-linked genes located on the X and Y chromosome, respectively. In addition, multiple mutations in both the extracellular domain (ECD) and the intracellular domain (ICD) of NL-4X have been shown to associate with ASDs. Interestingly, NL-4X and NL-4Y are highly conserved with only eight amino acid differences in the ECD and five in the ICD. Although NL-4X has been shown to be phosphorylated by protein kinase C (*Bemben et al., 2015*), there are no studies on phosphorylation of NL-4Y. Here, using an *in vitro* kinase assay, we report that phosphorylation of NL-4Y by PKC is significantly weaker than NL-

4X. Furthermore, using different kinases in conjunction with mass spectrometry, we show that NL-4X and NL-4Y are phosphorylated at different residues. Taken together, our results suggest that NL-4X and NL-4Y are regulated in a fundamentally different manner, and through a better investigation of the sex-linked isoforms of NLs, we hope to further understand the sex bias associated with ASDs.

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Poster

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Thomas Jefferson University Programmatic Theme Team Award

Title: Functional differences between NLGN4X and NLGN4Y

Authors: R. HODGE[1], *M. B. DALVA[2,1];
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Abstract: Correct assembly and function of neural circuits relies on the proper formation and maturation of a diverse array of synapses. Synapse formation requires contact between pre- and postsynaptic neurons and is thought to be induced by interactions between synaptic cell adhesion molecules. One of the most widely studied pairs of synaptic cell adhesion molecules are the presynaptic neurexins (NRXN) and postsynaptic neuroligins (NLGN). Binding of NRXN to NLGN is crucial for recruitment and clustering of pre- and postsynaptic complexes and synaptic transmission. Nearly all vertebrates encode four NLGNs and, while NLGN1-3 are well characterized, little is known about the localization and function of NLGN4. In most mammals NLGN4 resides on the X chromosome. Interestingly, humans and higher order primates carry a fifth male-specific NLGN located on the Y chromosome. Having approximately 98% sequence homology to NLGN4X, this additional NLGN is often referred to as NLGN4Y. With both X and Y NLGN4 isoforms in humans, males and females naturally express a different composition of NLGN4, the functional implications of which are as yet unexplored. Mutations in NLGN4X and NLGN4Y have been identified in individuals as a cause of several disorders characterized by

synaptic dysfunction such as autism spectrum disorder (ASD). Furthermore, in XYY males there is a correlation between NLGN4Y expression levels and an increased risk for ASD. Despite these observations, a role for NLGN4 at synapses has not yet been established. This largely overlooked area has prompted us to investigate the function of NLGN4 and potential differences in function between NLGN4X and NLGN4Y. Using an established fluorescent-based in vitro affinity assay, we have examined the affinity of NLGN4X and NLGN4Y for NRXN1-β and determined whether binding to NRXN1-β is altered upon coexpression of NLGN4X and NLGN4Y. To monitor the functional impacts of coexpression, we have used a heterologous co-culture assay to determine whether or not potential differences in affinity lead to differences in synaptogenesis between NLGN4X and NLGN4Y when expressed individually or together. Additionally, it is unclear whether NLGN4 is located at excitatory synapses, inhibitory synapses, or both. Towards this end, have examined the impact of exogenous expression of human NLGN4X and NLGN4Y in primary rat cortical neurons on excitatory versus inhibitory synaptic organization. Taken together, our studies may shed light on a potential role for NLGN4 at synapses and could uncover functional differences between NLGN4X and NLGN4Y.

Disclosures: R. Hodge: None. **M.B. Dalva:** None.

Poster

589. Postsynaptic Organization and Structure II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 589.13/F32

Topic: B.07. Synaptic Transmission

Support: Auckland Medical Research Foundation

Title: Regulation of synaptic glutamate receptor distribution in mutant huntingtin expressing neurons

Authors: W. AMBROZIAK, *J. M. MONTGOMERY;
Univ. of Auckland, Auckland, New Zealand

Abstract: Huntington's disease (HD) is a neurodegenerative genetic disorder caused by expansion of a CAG repeat tract in the *HTT* gene. Although striatal degeneration that causes late-onset movement impairment is the most pronounced, psychiatric and cognitive symptoms appear earlier in HD progression and have been proposed to originate from the prefrontal cortex and hippocampus. N-methyl-D-aspartate receptors (NMDARs) are glutamate receptors underlying excitatory synaptic transmission and plasticity. Electrophysiological studies on transgenic HD mouse models suggest that extrasynaptic NMDARs drive neurodegeneration by triggering cell

death-associated signalling pathways via pCREB shutoff. Activity of synaptic NMDARs increases pCREB levels and promotes neuronal survival. To study the role of extrasynaptic NMDARs in HD hippocampal pathogenesis, we transfected hippocampal cultured neurons with normal (25 CAG repeats) or mutant Huntingtin protein (mHTT; 97 CAG repeats). To visualise synaptic vs extrasynaptic NMDARs, we applied dSTORM imaging and demonstrated that in hippocampal neurons expressing mHTT the NMDAR localisation is significantly shifted towards the extrasynaptic sites. To determine whether the receptor shift to extrasynaptic sites can be rescued, we focussed on Synapse-Associated Protein-97 (SAP97), a major synaptic protein responsible for distribution of glutamate receptors. Alternative splicing of SAP97 gives rise to two N-terminal isoforms, alphaSAP97 and betaSAP97, the former acting as a scaffolding protein in the synapse and the latter participating in receptor segregation and trafficking. Our data show that overexpression of either SAP97 isoform alters NMDAR distribution in mHTT neurons. However, while alphaSAP97 reinstates the normal synaptic receptor distribution, betaSAP97 upregulates the synaptic NMDARs beyond the level of the controls. This effect is only seen in mHTT-expressing cells and not in controls. Moreover, alphaSAP97 is down-regulated in the hippocampus and striatum of YAC128 mouse HD model. Our data suggest that a change in balance between SAP97 isoform expression levels is a part of the HD pathogenesis, and that restoring the isoform balance has the potential to return the balance of synaptic and extrasynaptic receptors

Disclosures: **W. Ambroziak:** None. **J.M. Montgomery:** None.

Poster

589. Postsynaptic Organization and Structure II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 589.14/F33

Topic: B.07. Synaptic Transmission

Title: Altered neuromuscular structure and function in sedentary adult mice

Authors: ***S. BARBAT**, R. ROBITAILLE;
Univ. De Montréal, Montreal, QC, Canada

Abstract: Sedentarity has been associated with muscle weakness and atrophy. Here, we investigated the alterations at the neuromuscular junction (NMJ), the synapse conveying the motor command from the brain to the muscle. We compared soleus muscles of sedentary adult mice (12 months) to age-matched active mice. NMJ structure was assessed by immunolabeling of pre-, post and glial elements while synaptic properties were studied using electrophysiological recordings. As expected, the body weight was larger in sedentary mice (51.6±1.0 vs. 44.8±1.0 g,

p=0.001) while soleus muscle mass was lower (19.0±0.4 vs. 21.2±0.4 mg, p=0.001). NMJs of sedentary mice were more fragmented than those of active mice (3.7±0.3 vs. 1.9±0.2 fragments, p=0.023) and their quantal content tended to be higher (3.8±0.3 vs 3.1±0.2, p=0.065). Furthermore, short-term synaptic potentiation following high frequency stimulation (60 Hz, 30 s) was smaller (9±5% vs. 36±4%, p=0.002). We hypothesise that the observed changes in NMJ properties are influenced by glial cells owing to their regulation of NMJ structure and function. Hence, sedentarity is associated with NMJ alterations that can lead to muscle weakness and atrophy.

Disclosures: **S. Barbat:** None. **R. Robitaille:** None.

Poster

589. Postsynaptic Organization and Structure II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 589.15/F34

Topic: B.07. Synaptic Transmission

Support: MH079407

Title: PDZ domain-containing protein FRMPD2 promotes synaptogenesis and synaptic activity

Authors: ***Y. HUO**;
Biol., Boston Univ., Boston, MA

Abstract: FRMPD2 is a FERM- and PDZ-domain containing protein highly expressed in the brain. In epithelia cells, FRMPD2 has been shown to have a unique polarized basolateral localization pattern, and is involved in the formation of tight junctions. FRMPD2 contains three PDZ domains, a structural feature shared among many synaptic proteins such as PSD-95. However, its role in neurons remains unknown. Our work shows that FRMPD2 is highly expressed during brain development. In cultured neurons, we find that FRMPD2 is enriched at the synaptic domain. Over-expression of FRMPD2 increases synapse density and alters spine morphology, while a down regulation in synapse formation is observed after siRNA-mediated knockdown of FRMPD2. Its role in synaptogenesis is dependent on neuroligin 1. The interaction of FRMPD2 with F-actin recruits actin to the spine as a scaffold facilitating spine growth. In line with its role in synapse formation, expression of FRMPD2 results in an increase in mEPSC amplitude and frequency. This data reveals FRMPD2 as a novel synaptic PDZ molecule that plays a critical role in neural circuit establishment and synaptic transmission.

Disclosures: **Y. Huo:** None.

Poster

589. Postsynaptic Organization and Structure II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 589.16/F35

Topic: B.07. Synaptic Transmission

Support: NIH Grant R37

Title: Neuronal pentraxin receptor induces postsynaptic specialization

Authors: ***S. LEE**[1], M. WEI[2], C. ZHANG[2], T. C. SÜDHOF[1,3];
[1]Mol. and Cellualr physiology, Stanford Univ., Stanford, CA; [2]Peking Univ., Beijing, China;
[3]Howard Hughes Med. Inst., Stanford, CA

Abstract: Neuronal pentraxins (NPs) act as synaptogenic molecules via their interaction with AMPA-type glutamate receptors (GluRs). The neuronal pentraxin receptor (NPR) is a unique NP in that it possesses a transmembrane domain and can be tethered to the synaptic membrane, but how it regulates synaptogenesis is unclear. We observed that membrane-tethered NPR is solely sufficient for the recruitment of both excitatory and inhibitory postsynaptic specialization. It works as a typical synaptic adhesion molecule through binding with its postsynaptic adhesion partners, GluRs in excitatory synapse. NPR binding to GluR1 enhances its channel activity and the blocking AMPAR activity in neurons inhibits NPR-dependent postsynaptic specialization. On the other hand, NPR also induces in inhibitory postsynaptic specialization. Blocking GABA channel activity reduces the NPR-dependent postsynaptic specialization. These results suggest that NPR promotes synaptogenesis through both AMPA and GABA channels in excitatory and inhibitory synapses, respectively.

Disclosures: **S. Lee:** None. **M. Wei:** None. **C. Zhang:** None. **T.C. Südhof:** None.

Poster

589. Postsynaptic Organization and Structure II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 589.17/F36

Topic: B.07. Synaptic Transmission

Title: Synaptic adhesion molecule IgSF11 interacts with PSD-95 and regulates AMPA receptor-mediated synaptic transmission and plasticity

Authors: *S. JANG;
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Abstract: Synaptic adhesion molecules regulate synapse development and plasticity through mechanisms including trans-synaptic adhesion and recruitment of diverse synaptic proteins. We report here that the immunoglobulin superfamily member 11 (IgSF11), a homophilic adhesion molecule preferentially expressed in the brain, is a novel and dual-binding partner of the postsynaptic scaffolding protein PSD-95 and AMPAR glutamate receptors (AMPARs). IgSF11 requires PSD-95 binding for its excitatory synaptic localization. In addition, IgSF11 stabilizes synaptic AMPARs, as shown by IgSF11 knockdown-induced suppression of AMPAR-mediated synaptic transmission and increased surface mobility of AMPARs, measured by high-throughput, single-molecule tracking. IgSF11 deletion in mice leads to suppression of AMPAR-mediated synaptic transmission in the dentate gyrus and long-term potentiation in the CA1 region of the hippocampus. IgSF11 does not regulate the functional characteristics of AMPARs, including desensitization, deactivation, or recovery. These results suggest that IgSF11 regulates excitatory synaptic transmission and plasticity through its tripartite interactions with PSD-95 and AMPARs.

Disclosures: S. Jang: None.

Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 590.01/F37

Topic: B.09. Intrinsic Membrane Properties

Support: NIH Grant R01MH095995 (FL)

NIH Grant R01DA029091-01 (TG)

Title: GSK3 signaling regulates intrinsic firing and persistent sodium current in medium spiny neurons of the nucleus accumbens

Authors: F. SCALA[1,2], *M. N. NENOV[1], E. CROFTON[1], Y. ZHANG[1], N. WILDBURGER[1], B. CHESSON[1], T. JAMES[1], M. ALSHAMMARI[1,3], T. ALSHAMMARI[1,3], C. LITCHI[1], J. RUDRA[1], M. D'ASCENZO[2], T. GREEN[1], F. LAEZZA[1];
[1]Univ. of Texas Med. Br., Galveston, TX; [2]Inst. of Human Physiology, Univ. Cattolica, Rome, Italy; [3]Col. of Pharmacy, King Saud Univ., Riyadh, Saudi Arabia

Abstract: Studies indicate that enriched environmental conditions (EC) can exert protective effects against depression and addiction. Using unbiased transcriptomic analysis in the nucleus accumbens (NAc) of EC rats mRNAs coding for glycogen synthase kinase 3 (GSK3) and the voltage-gated Na^+ channel Nav1.6 were identified as part of a protective genetic program against depression and addiction. With a combination of *in vivo* Adeno-Associated Virus (AAV) vector gene silencing and acute brain slice patch-clamp electrophysiology in the nucleus accumbens we found that silencing of either GSK3 or Nav1.6 with selective AAV short hairpins leads to a reduction in maximum firing frequency and persistent Na^+ currents in medium spiny neurons (MSN). On the other hand, MSN in GSK3 knock-in mice expressing GSK3 isoforms resistant to inhibitory PI3K/AKT phosphorylation exhibited an opposite phenotype with increased firing and augmented persistent Na^+ currents. To provide an *in vitro* mechanistic model, we characterized the effect of GSK3 inhibition on Nav1.6-encoded currents demonstrating that suppression of the kinase activity decreases Nav1.6 currents. Using *in vitro* phosphorylation and mass-spectrometry we identified a GSK3 phosphorylation site on Nav1.6 and designed a 15-mer peptide flanking this site along with alanine mutants to selectively reverse hyper-excitability of MSN in GSK3 knock-in mice. Altogether, these results provide a novel molecular model of how changes in GSK3 signaling might result into functional neurocorrelates associated with depression and addiction leading to potential new therapeutics targeting posttranslational modifications within the GSK3 intracellular cascade.

Disclosures: **F. Scala:** None. **M.N. Nenov:** None. **E. Crofton:** None. **Y. Zhang:** None. **N. Wildburger:** None. **B. Chesson:** None. **T. James:** None. **M. Alshammari:** None. **T. Alshammari:** None. **C. Litchi:** None. **J. Rudra:** None. **M. D'Ascenzo:** None. **T. Green:** None. **F. Laezza:** None.

Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 590.02/F38

Topic: B.09. Intrinsic Membrane Properties

Support: NIH Grant MH071739

NIH Grant GM058234

Title: Excitation-alternative splice coupling (E-AS coupling) supports homeostatic regulation of neuronal excitability

Authors: *B. LI, B. SUUTARI, R. W. TSIEN;
Neurosci. Institute, Tsien Lab., New York Univ. Med. Ctr., New York, NY

Abstract: In contrast to the positive feedback in Hebbian plasticity, homeostatic plasticity maintains the information transfer capacity of brain circuit by negative feedback regulation. We have clarified the signaling mechanisms by which reduced neuronal activity leads to the changes in alternative splicing and thereby contributes to homeostatic regulation of neuronal excitability. Chronic inactivity (48 hr TTX) prolongs action potential (AP) duration, in large part via alternative splicing of BK channels. Inclusion of exon 29 (E29) in BK channel dropped to ~50%, while total BK mRNA remained unchanged; in turn, BK channels lacking E29 exhibited lower channel activity and led to wider APs than BK channels including E29. Moreover, overexpression of E29-included BK channel completely blocked TTX-induced AP prolongation, confirming that TTX-induced E29 skipping contributes to homeostatic regulation of excitability. Alternative splicing of E29 was regulated by Nova-2, which directly binds to the intron downstream of E29. Overexpression of Nova-2 drove E29 inclusion in E29 minigene-transfected N2A cells; knockdown of Nova-2 strongly reduced E29 inclusion in cortical neurons, which could be reversed by shRNA-resistant Nova-2. These data suggest that Nova-2 is both sufficient and necessary for E29 inclusion. Chronic inactivity triggered nuclear export of Nova-2 in a Ca_V1-CaMKK-CaMKIV-dependent manner: TTX-induced Nova-2 translocation and regulation of E29 splicing was prevented upon blocking Ca_V1 channels by nimodipine, CaMKs by KN-93, CaMKK by STO-609 or upon buffering of nuclear CaM by CaMBP4(nu). Indeed, 48 hr TTX induced activation of cytosolic CaMKII and nuclear CaMKIV, which directly binds to and phosphorylates Nova-2; phosphomimetic mutations led to Nova-2 translocation. These events appear to be linked by TTX-induced translocation of CaMKK2, and apparently CaM shuttling to the nucleus. Knockdown of CaMKK2 expression blocked TTX-induced E29 splicing. We confirmed the involvement of Nova-2 and BK channel splicing in an *in vivo* model of homeostasis, mice undergoing monocular deprivation. 5 d monocular deprivation led to Nova-2 displacement from the nucleus and decreased E29 splicing in the visual cortex contralateral to the deprived eye, consistent with findings in cultured neurons. Thus, E-AS coupling contributes to homeostatic regulation of neuronal excitability *in vivo* as well as *in vitro*.

Disclosures: B. Li: None. **B. Suutari:** None. **R.W. Tsien:** None.

Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 590.03/F39

Topic: B.09. Intrinsic Membrane Properties

Support: NIA Grant R03 AG042814-02

Title: Neurons in rat retrosplenial cortex are intrinsically heterogeneous in firing type and morphology

Authors: *A. N. NYE[1], J. A. TUMA[2], J. R. MOYER, Jr.[2];
[2]Psychology, [1]Univ. of Wisconsin Milwaukee, Milwaukee, WI

Abstract: Retrosplenial cortex plays a vital role in the learning and memory of temporal, spatial, and contextual information. Additionally, recent data suggest that retrosplenial cortex is also involved in multiple aspects of trace fear conditioning, including memory for the CS, the context, and extinction learning. Unfortunately, few studies have investigated the intrinsic electrophysiological properties of retrosplenial cortical neurons. In the current study, coronal brain slices were prepared from experimentally naïve adult F344 rats. An upright microscope equipped with infrared DIC optics was used for visualizing the neuron and patch electrode, and whole-cell recordings were made from neurons located throughout different layers of granular retrosplenial cortex, also known as Brodman's area 29. Electrodes included biocytin in order to confirm laminar location of neurons, and obtain detailed morphological analyses. Data from 76 neurons revealed several distinct firing patterns, which included afterdepolarizing (ADP) regular spiking, double spiking, late spiking, and fast spiking. ADP-regular spiking neurons fired single action potentials with a pronounced afterdepolarization in response to a just-suprathreshold current injection. In contrast, double spiking neurons fired a doublet. In response to a just-suprathreshold current injection, late spiking neurons uniquely delayed their firing until near the end of the 1-sec current injection (mean spike latency ~807 ms), as compared to neurons from all other firing types (mean spike latency ~403 ms). Further experiments utilizing CNQX (AMPA receptor antagonist) and D-AP5 (NMDA receptor antagonist) revealed that the distinct firing patterns are intrinsic to the neurons and not due to glutamatergic synaptic input. Initial analyses has revealed morphological differences between late spiking neurons and both ADP-regular spiking and double spiking neurons. Ongoing experiments are investigating the specific ion channels contributing to neuronal firing patterns. These studies will contribute important physiological and morphological data and lay the foundation for understanding how retrosplenial cortex contributes to learning and memory as well as aging-related learning deficits. **Funding: NIA grant**

Disclosures: **A.N. Nye:** None. **J.A. Tuma:** None. **J.R. Moyer:** None.

Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 590.04/F40

Topic: B.09. Intrinsic Membrane Properties

Support: CNPq

Funcap

Title: Essential oil of Ocimum basilicum L and (-)linalool blocks the excitability of rat sciatic nerve

Authors: *F. W. FERREIRA[1], A. M. VENANCIO[2], K. S. SILVA-ALVES[1], H. C. PIMENTEL[2], M. M. LIMA[2], M. F. D. SANTANA[2], P. B. ALVES[2], G. B. D. SILVA[2], J. H. LEAL-CARDOSO[1], M. MARCHIORO[2];
[1]State Univ. of Ceará, Fortaleza, Brazil; [2]Federal Univ. of Sergipe, Aracaju, Brazil

Abstract: The racemate linalool and its levogyrus enantiomer, [(-)LIN] are present in many essential oils and possess several pharmacological activities, such as antinociceptive and anti-inflammatory. In this work it was studied the effects of essential oil obtained from the cultivation of the *Ocimum basilicum* (EOOb) derived from Germplasm Bank rich in (-)LIN content in the excitability of peripheral nervous system. We used rat sciatic nerve to investigate the EOOb and (-)LIN effects on neuron excitability and it was used the extracellular recording technique to register the compound action potential (CAP). EOOb and (-)LIN blocked the CAP in a concentration-dependent way and these effects were reversible after washout. EOOb blocked positive amplitude of 1st and 2nd CAP components with IC_{50} of 0.38 ± 0.2 and 0.17 ± 0.02 mg/mL, respectively. For (-)LIN, these values were 0.23 ± 0.07 and 0.13 ± 0.02 mg/mL. Both components reduced the conduction velocity of CAP and the 2nd component seems to be more affected than 1st component. In conclusion EOOb and (-)LIN inhibited the excitability of peripheral nervous system in a similar way and potency, revealing that the effects of EOOb on excitability is due to the presence of (-)LIN on the essential oil.

Disclosures: F.W. Ferreira: None. **A.M. Venancio:** None. **K.S. Silva-Alves:** None. **H.C. Pimentel:** None. **M.M. Lima:** None. **M.F.D. Santana:** None. **P.B. Alves:** None. **G.B.D. Silva:** None. **J.H. Leal-Cardoso:** None. **M. Marchioro:** None.

Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 590.05/F41

Topic: B.09. Intrinsic Membrane Properties

Support: R01NS036692

R01NS052634

Title: Reduced labeling of Perineuronal Nets and decreased Parvalbumin Neurons in the Peritumoral Cortex of a Glioma mouse model

Authors: *B. P. TEWARI[1], S. L. CAMPBELL[2], H. SONTHEIMER[3];
[1]Glial Biol. in Health, Dis. and Cancer Ctr., [2]Ctr. for Glial Biol. in Health, Disease, and Cancer, Virginia Tech. Carilion Res. Inst., Roanoke, VA; [3]Ctr. for Glial Biol. in Health, Disease, and Cancer, Virginia Tech. Carilion Res. Inst., Ronaoke, VA

Abstract: Glioma associated epileptic seizures have been attributed to the imbalance of excitatory and inhibitory neurotransmission. Peritumoral niche in the cerebral cortex exhibits unique physiological properties including decreased parvalbumin expressing interneurons and lower expression of KCC2 leading to abnormally high intracellular chloride thereby rendering GABA responses excitatory. Perineuronal nets (PNNs) are specialized extracellular matrix structures surrounding parvalbumin expressing interneurons and shown to protect neurons from oxidative stress and also possess ion sorting properties. Gliomas secrete matrix metalloproteinase which degrade extracellular matrix of brain parenchyma thereby facilitating glioma spread and invasion. Using a clinically relevant mouse model of glioma, we studied the changes in PNNs and the physiology of parvalbumin expressing interneurons that are associated with PNNs in peritumoral cortex. Immunohistochemical staining of PNNs by Wisteria floribunda agglutinin (WFA), revealed a progressive degradation of PNNs in the peritumoral region. Moreover, parvalbumin expressing interneurons also exhibited progressive decrease in numbers. Electrophysiological recordings of the remaining parvalbumin interneurons that are associated with PNNs exhibited significantly lower action potential firing rates compared to that of contralateral hemisphere. Neurons which were not associated with PNNs in the same peritumoral area, showed higher firing rates compared to that of contralateral hemisphere. Overall our results suggest that the alteration of peritumoral PNNs and the firing properties of their associated parvalbumin expressing interneurons is a potential mechanism that could contribute to decreasing inhibitory neurotransmission in peritumoral hyperexcitability.

Disclosures: **B.P. Tewari:** None. **S.L. Campbell:** None. **H. Sontheimer:** None.

Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 590.06/F42

Topic: B.09. Intrinsic Membrane Properties

Support: MRC 4-Year PhD Studentship

Wellcome Trust

Title: Cholinergic-dependent intrinsic homeostatic plasticity in a subset of CA3 neurons

Authors: *C. J. PUHL, W. WEFELMEYER, J. BURRONE;
Ctr. for Developmental Neurobio., King's Col. London, London, United Kingdom

Abstract: Neurons respond to long-lasting perturbations of activity by adjusting their properties to stabilise their firing. In the hippocampus modulatory signals induce oscillations that drastically alter the ongoing activity in the network.
Here, we studied how increases in network activity induced by activation of cholinergic pathways modulate the intrinsic firing properties of hippocampal neurons.
Acute treatment of rat organotypic hippocampal slices with carbachol (CCh), an acetylcholine receptor agonist, led to long-lasting gamma oscillatory behaviour observed in population spike recordings of CA3 pyramidal neurons. After long-term (48hrs) treatment with CCh, whole-cell patch-clamp recordings revealed two different populations with strikingly different response properties. Whereas most (70%) of CA3 cells showed no changes in intrinsic excitability, a subset of cells (30%) showed a dramatic reduction in excitability, characterised by only firing single action potentials in response to 500ms current injections and a large decrease in their input-output curve. We also observed a decrease in input resistance (175.51±16.91 MΩ in control to 122.65±9.83 MΩ in single spiking; p=0.0164)., as well as an increase in current density threshold (from 1.63±0.27 pA/pF to 3.88±0.45 pA/pF in single-spiking neurons; p=0.0003) . Pharmacological blockade of the Kv7/M-current normalised their input resistance and allowed these neurons to fire repetitively, in a manner similar to control cells. We are currently characterising the dendritic morphology of this subset of cells and testing the idea that long-term plasticity of Kv7 channels may be responsible for this form of hyperadaptation in CA3 neurons.

Disclosures: **C.J. Puhl:** None. **W. Wefelmeyer:** None. **J. Burrone:** None.

Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 590.07/F43

Topic: B.09. Intrinsic Membrane Properties

Support: NIH Grant GM076990

Title: Transcriptomic correlates of brain-wide electrophysiological diversity

Authors: *S. TRIPATHY, D. TEBAYKIN, B. LI, O. MANCARCI, L. TOKER, P. PAVLIDIS; Ctr. for High-throughput Biol., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Despite thousands of studies characterizing electrophysiological diversity of cell types throughout the brain, the relationship between neuronal biophysics and gene expression remains poorly understood. Here, by combining databases on neuron type specific transcriptomics and electrophysiology (neuroexpresso.org and neuroelectro.org), we present a proof-of-concept approach for relating brain-wide electrophysiological diversity to gene expression. Specifically, we identified 865 genes whose expression levels were significantly correlated with neuronal phenotypic diversity (FDR < 0.1); for example, Hcn3, Gabrd, and Mef2a correlated with resting potential, and Nkain1, Calb2, and Kcnab1 correlated with input resistance. Moreover, the majority of these gene-electrophysiology correlations (76%) replicated using an independent Allen Institute dataset on single cell RNAseq and electrophysiology (celltypes.brain-map.org). In ongoing work, we are validating whether these gene-electrophysiology correlations reflect true causal relationships, in part, by assessing the consistency of these correlations using previous literature on gene knockout models and ion channel specific pharmacology.

Disclosures: S. Tripathy: None. **D. Tebaykin:** None. **B. Li:** None. **O. Mancarci:** None. **L. Toker:** None. **P. Pavlidis:** None.

Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 590.08/F44

Topic: B.09. Intrinsic Membrane Properties

Title: Long-term modulation of excitability by NMDA receptor signaling in cerebellar stellate cells

Authors: *R. ALEXANDER, D. BOWIE;
McGill Univ., Montreal, QC, Canada

Abstract: The action potential (AP) is a fundamental signaling unit used by neurons to communicate across distance. The AP is generated by a complex interplay of several voltage-gated ion channel families, including Na+ and K+ channels, which determine the threshold and frequency of firing rates. We have observed a long-term increase in cerebellar stellate cell excitability by signaling of NMDA receptors that modulates both Na+ and K+ channel activity. Local application of NMDA induced a persistent increase in spontaneous action current frequency during cell-attached electrophysiological recordings. During whole-cell current clamp recordings stellate cells exhibited a time-dependent increase in evoked AP frequency and hyperpolarization of spike threshold, both of which were eliminated by pharmacological block of CaMKII. Voltage clamp experiments revealed that CaMKII inhibition eliminates the development of a putative TTX-resistant Na+ current as well as an outward K+ current. Our work provides insight into the role of NMDAR-dependent signaling in regulating inhibitory neuronal circuits of the cerebellum.

Disclosures: R. Alexander: None. **D. Bowie:** None.

Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 590.09/F45

Topic: B.09. Intrinsic Membrane Properties

Title: Cannabinoid receptor 2 induced modulation of spatio-temporal firing dynamics in dopamine neurons of the ventral tegmental area

Authors: *H. J. STRATTON[1], J. WU[2];
[2]Neurol. Res., [1]Barrow Neurolog. Inst., Phoenix, AZ

Abstract: The Ventral Tegmental Area is an important midbrain nucleus containing dopamine (DA) neurons that project to and receive projections from a variety of cortical and sub-cortical targets implicated in mediating a range of complex behavioral responses. Recently, cannabinoid receptor 2 has been isolated in neural tissue and functionally identified within the VTA, where it influences DA neuron firing activity. The CB2 receptor is a G-Protein coupled receptor bound to

internal Gi/o elements that can alter cellular excitability through modifications in potassium conductance and changes to intracellular calcium permeability and release. The endogenous ligands for CB2 act in a retrograde fashion upon presynaptic terminals that synapse on either GABAergic or DA neurons of the VTA. This retrograde signaling behaves primarily as a localized inhibitory mediator of cellular excitability through presynaptic attenuation in an input dependent fashion. The distribution of CB2 proteins within the VTA is currently not well known, but it is thought that these receptors are not homogeneous across synapses and instead are lightly spread across very specific subregions corresponding to particular neuronal microcircuits. In this study, we examined extracellular potentials recorded from the VTA using planar microelectrode arrays in acute slices prepared from C57BL/6J mice and corresponding CB2 knockout mice. The potentials recorded at a total of 64 sites spread across the entirety of the VTA were analyzed for local field potential content as well as single-unit spiking activity. Signal processing was performed offline and a differential threshold method was applied to identify spike events followed by a clustering algorithm, which analyzed waveform structure using principal components. This method allows for the identification of spike source neurons with high levels of certainty in order to characterize particular neuronal phenotypes across channels and across slices. Changes in the local field potential oscillation and spiking activity were observed between C57 and KO animals with perfusion of 1 micro molar carbachol to elicit higher baseline firing rates. In addition, JWH-133 and AM-630, CB2 receptor specific agonist and antagonist respectively, were applied to the bath in order to characterize effects of CB2 receptor activation on the spatial activity pattern recorded across the 8x8 grid of electrodes in the planar array. As expected, variations in power spectra and bursting characteristics were observed across medial as compared to lateral regions of the VTA indicating distinct populations of neurons that respond in a unique manner to CB2 modulation.

Disclosures: **H.J. Stratton:** None. **J. Wu:** None.

Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 590.10/F46

Topic: B.09. Intrinsic Membrane Properties

Support: Norwegian Research Council

The Human Brain Project

Title: Kv7/M-current function in axons: high-pass filtering of plateau potentials promotes full action potentials, also during cholinergic activation

Authors: *J. F. STORM, R. MURPHY, P. MATEOS-APARICIO;
Univ. of Oslo, Oslo, Norway

Abstract: Axons are equipped with Kv7/KCNQ/M potassium channels whose main function remains unknown. Here we propose that they perform high-pass filtering of depolarizing plateau potentials originating in dendrites and soma, to prevent inactivation of axonal Na^+ channels, thus promoting efficient axonal generation and propagation of full amplitude spikes throughout plateau depolarizations.Using patch clamp recordings from distal mossy fiber boutons (MFBs) in rat hippocampal slices, and the Kv7/M channel blocker XE991, we have confirmed (Alle..Storm, SfN Abst 42.2, 2009) that these axon terminals have a large Kv7/M-current (I_M) that causes local resonant filtering of subthreshold signals in the theta frequency range, affects summation of subthreshold synaptic potentials ("EPreSPS"; Alle & Geiger, Science 2006) and generates a "medium after-hyperpolarization" (mAHP), but contributes almost nothing to spike repolarization. Computational modelling reproduced these results and showed that the axonal I_M can also perform resonant filtering of subthreshold signals during propagation from soma to terminals; but this is weak except when the axon is depolarized. Long-lasting somatic plateau depolarizations activated the axonal I_M, which then strongly attenuated the depolarizations, preventing it from spreading along the axon, promoting axonal spikes during the plateau, by preventing Na^+ channel inactivation. (Apostolides et al., Neuron 2016, found that axonal K^+ channels can do this.) Our model showed that the voltage-dependence and slow kinetics of I_M makes it ideally suited for such high-pass filtering.
Acetyl choline (ACh) can suppress I_M and is essential for cortex and forebrain activation during conscious wakefulness and REM sleep. ACh promotes somato-dendritic depolarizing plateaus by suppressing K^+ currents, including I_M, and enhancing inward currents. But if I_M is suppressed also in the axon, it cannot perform its high-pass filtering function during cholinergic plateaus. Hence, we hypothesize that the distal axonal IM is resistant to cholinergic modulation. Indeed, our patch clamp recordings from distal MFBs showed that the axonal I_M was resistant to both muscarine (n=4) and oxotremorine (n=4) that block the somatic I_M.
We conclude that the axonal Kv7/M-current (I_M) perform filtering of depolarizing plateau potentials originating in dendrites and soma, thus promoting efficient axonal spike generation and propagation throughout the depolarizations, and that this function is maintained during cholinergic activation, thus presumably during conscious wakefulness. We propose that this is the main function of the axonal Kv7/M-current.

Disclosures: J.F. Storm: None. **R. Murphy:** None. **P. Mateos-Aparicio:** None.

Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 590.11/F47

Topic: B.09. Intrinsic Membrane Properties

Support: UCL IMPACT Studentship

a UCL Graduate School Doctoral Fellowship

UCL School of Pharmacy

ERC Starter Independent Grant (GA 260725 IRPHRCSTP, M.M.S.)

Title: The role of $Ca_v3.2$ Ca^{2+} channels in influencing Layer II Medial Entorhinal Cortex stellate cell activity along the dorsal-ventral gradient.

Authors: *A. P. TOPCZEWSKA[1], A. C. DOLPHIN[2], M. M. SHAH[1];
[1]Sch. of Pharm., [2]Neurosci. Physiol. and Pharmacol., UCL, London, United Kingdom

Abstract: Layer II (LII) Medial Entorhinal Cortex (MEC) stellate cell (SC) intrinsic membrane properties vary along the MEC dorso-ventral axis. This has been attributed partly to altered HCN and K^+ conductances[1,2]. The subthreshold active T-type $Ca_V3.2$ Ca^{2+} channels, though, are also expressed in the MEC[3]. $Ca_V3.2$ channels are known to influence neuronal excitability but their effects on the dorsal and ventral LII MEC SC properties remain unknown. To investigate this, we obtained acute entorhinal-hippocampal parasagital slices from $Ca_V3.2$ wildtype ($Ca_V3.2^{+/+}$) and null ($Ca_V3.2^{-/-}$) 5-8 week old mice and made electrophysiological recordings from dorsal and ventral LII MEC SC. $Ca_V3.2^{-/-}$ ventral neurons displayed significantly reduced input resistance but had little difference in resting membrane potential compared with $Ca_V3.2^{+/+}$ ventral neurons. Consequently, depolarizing steps generated fewer action potentials in $Ca_V3.2^{-/-}$ ventral SC than in their wildtype littermates. In contrast, dorsal $Ca_V3.2^{-/-}$ and $Ca_V3.2^{+/+}$ SC properties were similar. The Ca_v3 inhibitors, $NiCl_2$ (50 μM) and TTA-P2 (100 nM), also significantly reduced input resistance and excitability in $Ca_V3.2^{+/+}$ ventral neurons, whilst having little effect on $Ca_V3.2^{+/+}$ dorsal or $Ca_V3.2^{-/-}$ neurons. Our results suggest that $Ca_v3.2$ channels selectively affect the LII MEC ventral SC characteristics, thereby contributing to the intrinsic membrane gradient across the MEC dorsal-ventral axis.
References:
1. Garden, D.L. et al., Neuron, 60, 875-889, 2008.
2.Giocomo, L.M. & Hasselmo, M.E., J. Neurosci., 28, 9414-25, 2008.
3.Huang, Z. *et* al., Nat Neurosci, 14, 478-486, 2011.

Disclosures: **A.P. Topczewska:** None. **A.C. Dolphin:** None. **M.M. Shah:** None.

Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

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Program#/Poster#: 590.12/F48

Topic: B.09. Intrinsic Membrane Properties

Title: The intracellular calcium release from ryanodine channels controls action potential properties

Authors: L. ARTINIAN[1], H. GHABEL[2], L. ZHONG[3], S. ESTES[1], G. CYMBALYUK[2], *V. REHDER[3];
[1]Biol., [2]Neurosci. Inst., [3]Georgia State Univ., Atlanta, GA

Abstract: Spontaneous spiking activity plays critical roles in the function of many neurons and neuronal networks. The activities of several ion channels provide sub-threshold currents and drive repetitive spiking. *Helisoma trivolvis* buccal ganglia B5 neurons are tonically spiking neurons in situ and in single cell culture. Here we demonstrated that the tonic spiking activity of these neurons requires two major ionic currents, namely a persistent Na current (I_{Nap}) and a hyperpolarization-activated current (I_h).Both, I_{Nap} and I_h, and thus the spontaneously spiking activity of B5 neurons, were found to be under the control of calcium release from ryanodine receptors (RyR-CR), because the inhibition of RyR-CR significantly reduced I_{Nap} and I_h and silenced these neurons. Evoked spiking activity was affected by the reduction of I_{Nap} and I_h in response to inhibition of RyR-CR as well. Our experimental data showed that the inhibition of I_h, I_{Nap}, and RyR-CR narrowed action potential (AP) width. The mechanism of how the inhibition of I_{Nap} and I_h resulted in a decrease in AP's width was provided by mathematical modeling of spontaneous spiking activity. This model implicates an increase in interspike interval that inactivates Ca currents contributing to the width of APs. Taken together, our findings highlight the importance of RyR-CR in setting neuronal spiking activity via upregulation of the inward currents, I_{Nap} and I_h and, in turn, the regulation of the intracellular calcium concentration via modulation of AP width.

Disclosures: L. Artinian: None. **H. Ghabel:** None. **L. Zhong:** None. **S. Estes:** None. **G. Cymbalyuk:** None. **V. Rehder:** None.

Poster

590. Modulation of Neuronal Firing Properties I

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Topic: B.09. Intrinsic Membrane Properties

Support: INSPIRE Fellowship

Wellcome Trust-DBT India Alliance

Title: Role of slow temporal dynamics in reliability of stochastically driven neurons

Authors: *S. S. MOKASHE, S. NADKARNI;
Dept. of Biol., Indian Inst. of Sci. Educ. and Res., Pune, India

Abstract: Mutually inhibiting neurons is a common motif across many systems like Hippocampus, CPGs(Central Pattern Generators) and Olfaction. Their synaptic interaction ensures that they show alternating activity. The frequency of switching from an active to a quiescent period is a function of the biophysical properties of ion channels present in the neurons, synaptic interaction timescales, network properties, the stimulus and possibly channel fluctuations from small number of channels. Switching allows neurons to associate with different networks and coordinate patterns of activity that may be relevant for function. The frequency of switching dictates the sequential order of activity of neurons required for locomotion, for example in Lamprey. In this context, reliable switching might be a critical functional requirement. How do networks of mutually inhibiting neurons, a simple most functional module of switching, achieve this reliability despite a noisy framework and environment? We have developed a conductance based model of two mutually inhibiting neurons wherein inherent switching takes place via a potassium current, sAHP that is triggered by calcium ions. We investigate the role of slow buildup time scales associated with sAHP current in integrating calcium signal generated by stochastic opening of mediated by Voltage Dependent Calcium Channels(VGCCs). Furthermore we systematically study the effect of various sources of noise including channel conductance noise, synaptic noise and input noise on switching and robust generation of sequences.Our results show that switching frequency can be tuned with noise amplitude. Separately, there exists an optimal regime of noise where coefficient of variance of the inter-burst interval (IBI) is lowest, indicating precise switching. Our understanding on the effects of various sources of noise in this illustrative network motif is likely to be applicable to a wide variety of systems.

Disclosures: **S.S. Mokashe:** None. **S. Nadkarni:** None.

Poster

590. Modulation of Neuronal Firing Properties I

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Foundation Fighting Blindness to REA

Title: A novel role for very long chain fatty acids in brain function

Authors: *B. R. HOPIAVOURI[1], M.-P. AGBAGA[2], J. WILKERSON[3], R. BRUSH[4], A. HENDRICK[5], D. SAUNDERS[8], F. BHATTI[6], N. MANDAL[2], L. SZWEDA[9], V. AWASTHI[5], R. TOWNER[8], D. SHERRY[3], F. DEAK[7], R. E. ANDERSON[3];
[1]Oklahoma Ctr. for Neurosci., [2]Ophthalmology, [3]Cell Biol., [4]Opthalmology, [5]Dept. of Pharmaceut. Sci., [6]Col. of Medicine/Peds – Neonatology, [7]Geriatric Med., Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK; [8]Nuclear Magnetic Resonance Core Facility, [9]Aging & Metabolism Res. Program, Oklahoma Med. Res. Fndn., Oklahoma City, OK

Abstract: Purpose: ELOngation of Very Long chain fatty acids-4 (ELOVL4) is an elongase responsible for biosynthesis of very long chain (VLC; ≥C28) fatty acids; it makes VLC polyunsaturated fatty acids (VLC-PUFA) in retina and testes, and VLC saturated fatty acids (VLC-SFA) in skin and brain. A 2011 case study linked homozygous inheritance of the Stargardt's (STGD3) mutation in ELOVL4 with a central nervous system (CNS) phenotype in humans, including seizures, intellectual disability, spastic quadriplegia and death. We hypothesize that ELOVL4-synthesized VLC-SFA play an essential role in neural cell structure and function. **Methods:** We generated a successful animal model for STGD3/STGD3 inheritance (-/-). ELOVL4 localization within the CNS was determined in wild type mice (+/+) using immunofluorescence (IF) microscopy. Hippocampal lipids were analyzed by triple quadrupole mass spectrometry. Synaptic membrane fractionation was performed on baboon hippocampus for lipid analysis. Positron emission tomography (PET) was used to assess CNS uptake of fluorodeoxyglucose (FDG) in (-/-) mice. HPLC was used to assess intermediary metabolism in (-/-) mice. Hippocampal slices from (+/+) and (-/-) mice were subjected to spontaneous multi-

electrode array (MEA) recordings. Primary neuronal cultures from hippocampus of (+/+) and (-/-) mice were subjected to FM1-43 assessment of synaptic vesicle exocytosis rates. **Results:** Our STGD3/STGD3 mice recapitulate the human phenotype, developing seizures at P19 followed by death at P21. IF showed highest immunoreactivity in hippocampus. Hippocampal lipidomic analysis of (+/+) mice confirmed the presence of 28:0/30:0 in sphingolipids. Membrane fractionation of baboon hippocampus revealed enrichment of 28:0/30:0, but not VLC-PUFA, in synaptic vesicle membranes. PET imaging revealed a 3-fold increase in the amount of FDG uptake into the CNS of (-/-) mice. Metabolomic analysis revealed a significant increase in brain ATP levels in (-/-) mice. MEA recordings showed a significant increase in the amplitude and decrease in the inter-spike interval of action potentials in (-/-) vs (+/+) hippocampal slices. FM1-43 studies showed a significant increase in synaptic vesicle exocytosis rates in (-/-) vs (+/+) primary hippocampal neurons. **Conclusions:** This is the first study to demonstrate mutations in *Elovl4* causing a CNS phenotype in an animal model. The described studies suggest a neuron-specific role for VLC-SFA in the regulation of pre-synaptic synaptic function by impacting the rate of synaptic vesicle release.

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Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

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Topic: B.09. Intrinsic Membrane Properties

Support: NIAAA R01AA022821

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Russian Foundation for Basic Research (project no. 14-02-00916-a)

ANR-13-NEUC-0003 GABA

ANR-10-LABX-0087 IEC

ANR-10-IDEX-0001-02 PSL

Title: Optimizing synaptic inputs for phasic dopamine transients

Authors: *E. MOROZOVA[1], M. MYROSHNYCHENKO[2], D. ZAKHAROV[3], M. DI VOLO[4], B. GUTKIN[4], C. C. LAPISH[5], A. KUZNETSOV[5];
[1]Physics, [2]Program in Neurosci., Indiana Univ., Bloomington, IN; [3]Inst. of Applied Physics, Nizhni Novgorod, Russian Federation; [4]Ecole Normale Superieure PSL Univ., Paris, France; [5]Indiana Univ. - Purdue Univ., Indianapolis, IN

Abstract: Dopamine (DA) neurons play a central role in guiding motivated behaviors. However, complete understanding of computations these neurons perform to encode rewarding and salient stimuli is still forthcoming. Intrinsic properties of the neurons determine the neuron's response to stimuli, its synchronization properties and the computations it performs in the brain. We investigated the dynamical mechanisms underlying the excitability type of DA neurons and its regulation by synaptic currents using a biophysical model. We concentrated primarily on the influence of GABA input, considering that a substantial proportion of the synaptic drive to DA neurons comes from GABA neurons. Particularly, we considered the DA neuron excitability type, its dependence on the tonic coactivation of GABA with NMDA or AMPA receptors, and the effect of temporal organization of GABA inputs on the dynamics of DA neuron firing. DA neurons can switch from type I excitability to type II depending on the conductance composition. During balanced activation of GABAR and NMDAR, DA neurons exhibit low frequency tonic firing and type I excitability, whereas activation of AMPAR or elevation of GABAR reversal potential switches it to type II. Type I excitability might be important for achieving low basal DA concentration necessary for normal brain functioning and is perfect for calculation of reward prediction error, as type I neurons are best suited for coding stimulus intensity. Switching to type II excitability, however, enables robust transient DA release, partially due to evoked DA population synchrony in response to correlated inputs. Our investigations have revealed that the firing pattern of the DA neuron depends on the level of synchronization among GABA neurons. Synchronous GABA input enables an increase in DA neuron firing and bursting. Distinct from previous mechanisms, the increases were not based on lowered GABA firing rate or weaker hyperpolarization by the GABAR current. A mechanism of GABA-mediated increases in firing is based on a dynamical reduction the Ca^{2+} -dependent K^{+} currents. The biological relevance of predictions made by our model was validated by calculating cross-correlograms between pairs of simultaneously recorded putative VTA GABA neurons. We observed a large number of pairs (76 out of 197) that exhibit millisecond timescale synchrony, which is, according to our modeling results, more than necessary to achieve an increase in DA burstiness. Our results provide mechanistic understanding of the diverse mechanisms whereby GABA inputs regulate DA neuron burst firing.

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

Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 590.16/F51

Topic: B.09. Intrinsic Membrane Properties

Title: Bi-stable states allow neurons to generate highly irregular spike trains with weakly fluctuated inputs.

Authors: *R. HOSAKA;
Fukuoka Univ., Fukuoka, Japan

Abstract: The irregular firing of a cortical neuron is thought to result from a highly fluctuating drive that is generated by the balance of excitatory and inhibitory synaptic inputs. A previous study reported strange responses of the Hodgkin-Huxley neuron to the fluctuated inputs where an irregularity of spike trains is inversely proportional to an input irregularity. In the current study, we investigated the origin of these strange responses with the Hindmarsh-Rose neuron model, map-based models, and a simple mixture of interspike interval distributions. First, we specified the parameter regions for the bifurcations in the Hindmarsh-Rose model and confirmed that the model reproduced the strange responses in the dynamics of the saddle-node and subcritical Hopf bifurcations. For both bifurcations, the Hindmarsh-Rose model shows bistability in the resting state and repetitive firing state, which indicated that the bistability was the origin of the strange input-output relationship. Similarly, the map-based model that contained bistability reproduced the strange responses, while the model without bistability did not. These results were supported by additional findings that the strange responses were reproduced by mimicking the bistable firing with a mixture of two different interspike interval distributions. Decorrelation of spike trains is important for neural information processing. For such spike train decorrelation, irregular firing is key. Our results indicated that irregular firing can emerge from fluctuating drives, even weak ones, under conditions involving bistability. The strange responses therefore contribute to efficient processing in the brain.

Disclosures: R. Hosaka: None.

Poster

590. Modulation of Neuronal Firing Properties I

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Topic: B.09. Intrinsic Membrane Properties

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Title: STIM1 and neuronal excitability important for cerebellum-dependent learning

Authors: C. RYU, D. JANG, D. JUNG, *S. KIM;
Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: The dynamics of Ca^{2+} between the cytosol and intracellular stores has been considered as an important process for cellular mechanisms of learning and memory. Stromal interaction molecule 1 (STIM1) plays a pivotal role in maintaining Ca^{2+} store. However, its function in these mechanisms, including synaptic plasticity and neuronal excitability, remains obscure. Here, by using cerebellar Purkinje neuron-specific STIM1 knockout ($STIM1^{PKO}$) mice, we identify that STIM1-mediated Ca^{2+} dynamics contributes to the excitability of Purkinje neurons and cerebellum-dependent memory. Firing activity of STIM1-deleted Purkinje neurons is reduced with strengthened spike frequency adaptation, while their long-term synaptic plasticity is not affected. In cerebellum-dependent motor learning, $STIM1^{PKO}$ mice show impaired memory consolidation without defects in memory acquisition. Our findings suggest that the neuronal excitability regulated by STIM1 enables Purkinje neurons to transfer neuronal information properly for persistent memory.

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Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

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Program#/Poster#: 590.18/F53

Topic: B.09. Intrinsic Membrane Properties

Title: Serotonergic modulation of fast-spiking interneurons in medial prefrontal cortex

Authors: *J. ATHILINGAM, K. BENDER, V. SOHAL;
Neurosci., Univ. of California San Francisco, San Francisco, CA

Abstract: The prefrontal cortex is a highly evolved brain region that is responsible for directing higher order cognitive functions ranging from decision making to social cognition. Patients with schizophrenia exhibit marked deficits in prefrontal-dependent tasks such as working memory and rule shifting and also have alterations in a specific subtype of inhibitory interneuron in this region. Indeed, restoring the function of these fast-spiking interneurons (FSIs) can rescue behavioral deficits in mice that model aspects of this disease (Cho et al., 2015).
Most second-generation antipsychotic drugs target the neuromodulator serotonin (5HT) with high affinity. However, the effect of 5HT on fast-spiking interneurons in prefrontal cortex and how this might influence cortical information processing at the cellular and network levels and/or affect cognition is unknown.
Here, we use patch-clamp electrophysiology and optogenetics to demonstrate that 5HT acts on 5HT2A receptors to increase the intrinsic excitability and responses to input in FSIs by closing leak potassium channels. Further, we explore how serotonergic modulation of FSIs can influence synaptic integration and network oscillations. These findings can broadly inform our understanding of the role of neuromodulation in prefrontal function, and may provide insight into the etiology of schizophrenia as well as potential new avenues for rescuing prefrontal dysfunction in this disorder.

Disclosures: J. Athilingam: None. **K. Bender:** None. **V. Sohal:** None.

Poster

590. Modulation of Neuronal Firing Properties I

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Program#/Poster#: 590.19/G1

Topic: B.09. Intrinsic Membrane Properties

Support: NIH Grant DC04285

Title: Mechanisms regulating persistent spiking in rodent neocortical neurons *In vitro*

Authors: E. D. CUI, *B. W. STROWBRIDGE;
Case Western Reserve Univ., Cleveland, OH

Abstract: While all brain regions respond transiently to synaptic input, some brain circuits also generate long-lasting firing epochs following stimulation. The cellular mechanisms governing the dynamics of these persistent responses are not well understood. We examined this question using whole-cell patch clamp recording in rat temporal association neocortical slices exposed to low (2 μM) carbachol (CCh), a cholinergic receptor agonist previously demonstrated to promote persistent firing in a variety of cortical brain regions. We find evidence for multiple calcium-sensitive mechanisms regulating persistent activity. Chelating intracellular calcium ion with 10 mM 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) abolished persistent firing evoked by depolarizing step stimuli in regular spiking layer 5 pyramidal cells (PCs; N=6), likely reflecting a role for a prolonged calcium-sensitive depolarization underlying persistent spiking. However, the frequency of persistent firing was not maximal immediately following depolarizing step stimuli, when intracellular calcium concentration was likely maximal. Instead, the firing rate slowly increased over 22.6 sec (N=7). Increasing extracellular calcium levels from 2.5 to 4 mM decreased persistent firing frequency by 36%, measured 10 sec following offset of triggering depolarizing step (N=5). Both the slow ramp-up in persistent firing frequency and the inhibitory effect of enhanced calcium influx likely reflect critical roles for calcium-activated inhibitory conductances in modulating persistent firing. Consistent with this hypothesis, we found that bath application of the SK channel activator (NS309) decreased the duration of persistent firing following depolarizing step stimuli. With only CCh, 71% of regular spiking layer 5 PCs generated persistent responses that lasted at least 30 sec; the remaining 29% of recordings failed to generate any persistent activity. With SK currents enhanced with NS309, persistent firing spontaneously terminated before 30 sec in most (11/18) recordings. The duration of self-terminating persistent firing was graded with the degree of SK activation (2250 ms with 1 μM, N=5; 10700 ms with 0.5 μM NS309, N=6). These results suggest that the duration of persistent spiking responses can be modulating by calcium-activated potassium currents. Using this mechanism, individual neurons could regulate their calcium-sensitive responses to reliably encode different types of information over diverse timescales.

Disclosures: E.D. Cui: None. **B.W. Strowbridge:** None.

Poster

590. Modulation of Neuronal Firing Properties I

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Program#/Poster#: 590.20/G2

Topic: B.09. Intrinsic Membrane Properties

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NIH Grant MH076929

Title: Direct leptin action on hippocampal neuron excitability and behavioral responses

Authors: J. G. WANG[1], M. GUO[2], *X.-Y. LU[1];
[1]Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX; [2]Binzhou Med. Univ. Hosp. / IMND, Shandong, China

Abstract: We have previously shown that leptin target neurons within the hippocampus are largely restricted to the granule cell layer of the dentate gyrus (DG) and constitute a small percentage of DG granule cells that represent a subpopulation devoid of stress-induced activation (*Wang et al., 2015, Mol. Psychiatry 20:509-19*). Despite prior reports demonstrating leptin effects on excitability of hippocampal neurons, evidence is lacking for the direct effect of leptin on its target neurons. To determine whether leptin directly influences excitability of its target neurons expressing the leptin receptor (LepRb), we utilized the $Lepr^{Cre}$:tdTomato reporter mice that allowed us to precisely identify LepRb-expressing neurons. Whole-cell patch-clamp recordings were made from acute hippocampal slices of $Lepr^{Cre}$:tdTomato reporter mice. We found that leptin decreased intrinsic excitability of DG LepRb-expressing granule cells. The rheobase was increased, and the firing rate of action potentials was decreased by leptin. Furthermore, we examined whether selective inhibition of LepRb neurons in the DG affects depressive-like behaviors. AAV-DIO-hM4D(Gi)-mCherry (DREADD) was injected into the DG of Lepr-Cre transgenic mice, in which LepRb neurons specifically expressed hM4Di. We found that inhibition of DG LepRb neurons with DREADD induced antidepressant-like effects without affecting locomotor activity, consistent with the behavioral effects of intra-DG infusion of leptin. These results suggest that leptin induces direct inhibition of DG granule neurons and inhibition of DG leptin target neurons leads to antidepressant-like responses.

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Poster

590. Modulation of Neuronal Firing Properties I

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Topic: B.09. Intrinsic Membrane Properties

Support: Cnpq

Funcap

Capes

Title: Melatonin decreases neuronal excitability in a sub-population of DRG neurons in Wistar rats

Authors: K. OLIVEIRA-ABREU[1], K. S. SILVA-ALVES[1], F. W. FERREIRA-DA-SILVA[1], N. M. SILVA-DOS-SANTOS[1], F. G. D. AMARAL[2], A. C. CARDOSO-TEIXEIRA[1], *A. N. COELHO-DE-SOUZA[3], J. CIPOLLA-NETO[2], J. H. LEAL-CARDOSO[1];
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Abstract: Melatonin is a neurohormone mainly produced and secreted at night by the pineal gland. In the central nervous system, it has many effects related to physiological and pathological processes, such as circadian regulation, neuroprotection, antinociception and antioxidant activities. However, physiological effects of melatonin on the peripheral nervous system (PNS) remain poorly understood. Thus, this study investigated the effects of melatonin on excitability of a neuronal subpopulation (N_0 neurons) of intact dorsal root ganglion (DRG) from rats using intracellular recording technique in current clamp mode. Male Wistar rats were kept on a 12:12 h light/dark cycle with food and water available *ad libitum.* All animals used for electrophysiological experiments were sacrificed at Zeitgeber Time (ZT) 3 (lights-on at 06:00 h). Melatonin blocked the generation of action potentials (AP) in a concentration-dependent manner: 1 nM melatonin blocked AP generation in only one of 15 DRG neurons (6.6%). At concentrations of 10, 100 and 1000 nM, this blockade was 21.4% (6/28), 24% (6/25) and 30% (9/30), respectively. In neurons in which melatonin did not block the AP, there was an increase in rheobase. A hyperpolarizing effect of resting potential (E_m) was observed in these neurons. The magnitude of E_m hyperpolarization for melatonin at 10, 100 and 1000 nM was -1.70, -1.73 and -2.61 mV, respectively. Both 10 and 100 nM melatonin increased input resistance (R_{in}) values from 16.98 ± 2.33 to 27.54 ± 3.79 MΩ (10 nM; n = 22) and 14.13 ± 1.50 to 28.18 ± 3.73 MΩ (100 nM; n = 19). Additionally, melatonin significantly increased the peak AP amplitude and the maximal rate of both depolarization and repolarization. To determine the signal transduction pathway for melatonin in DRG neurons, qPCR analyses were performed for the

melatonin receptors MT_1 and MT_2. It was observed that DRG neurons express only MT_1 and that receptor expression peaks at ZT3. In conclusion, melatonin reduces excitability of N_0 DRG neurons in a concentration-dependent manner probably through the MT1 receptor. These type of neurons conveys information about mechanoception but also nociception information. Thus, excitability inhibition promoted by melatonin could act as a relevant mechanism of control of PNS.

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Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 590.22/G4

Topic: B.09. Intrinsic Membrane Properties

Support: TR-SFB134 to P.K.

CONNECT to P.K.

Fellowship of the Cologne Graduate School of Ageing Research to U.C.

We thank Helmut Wratil for outstanding technical assistance.

Title: Attempts to measure calcium currents in substantia nigra dopaminergic neurons

Authors: *U. COLLIENNE[1,2], A. C. KLEIN[1,2], S. HEß[1,2], S. BREMSER[1,2], P. KLOPPENBURG[1,2];
[1]Biocenter, Inst. For Zoology, Cologne, Germany; [2]Cologne Excellence Cluster on Cell. Stress Responses in Aging-Associated Dis. (CECAD), Univ. of Cologne, Cologne, Germany

Abstract: Midbrain dopaminergic (DA) neurons are categorized in three groups: the retrorubral area, the ventral tegmental area (VTA) and the substantia nigra pars compacta (SNpc). Morphologically, these groups are associated with two systems: the nigrostriatal pathway, which is mainly build by projections of the SNpc neurons and the mesocorticolimbic system, which arises primarily from the VTA neurons. Historically, the nigrostriatal pathway has been associated with the modulation of motor functions, while the mesocorticolimbic system was thought to be involved in motivation, reinforcement and reward-seeking behaviors. However, it becomes increasingly clear that SNpc and VTA neurons have overlapping projections and that

all three DA neuron subpopulation contribute to reward-related behaviors.
DA neurons are autonomous pacemakers. Synaptically isolated, they generate action potentials with a constant, very regular frequency. *In vivo*, this regular firing can be modulated by synaptic input leading to a burst firing pattern and a hyperpolarized, non-firing state. The resulting firing patterns determine the DA release in the projection areas and are correlated with the prediction and detection of rewards.
In SNpc neurons the autonomous pacemaking depends critically on the precise control of intracellular calcium dynamics. The calcium influx that is necessary to mediate pacemaking is thought to be generated by orchestrated activation of several voltage-activated calcium channels with specific functional properties. Especially L-type calcium channels are thought to mediate a substantial part of the calcium influx present during the pacemaker activity, because these channels are known to activate at sub-threshold membrane potentials. However, direct, quantitative and stable measurements of the voltage-activated calcium currents have been challenging due to 1) imperfect voltage control due to the complex morphology of SNpc neurons, 2) the presence of large outward current and 3) a rapid wash-out of the calcium currents. While the mean peak amplitudes typically reached -2.0 +/- 0.2 nA at the beginning of whole-cell patch clamp recordings in acute adult brain slice preparations, these calcium currents ran down within several minutes. Therefore, we aim to improve the recordings by optimizing the extra- and intracellular solution used for whole-cell measurements, and/or by combining several approaches including perforated patch clamp recordings and discontinuous single electrode voltage clamp.

Disclosures: U. Collienne: None. **A.C. Klein:** None. **S. Heß:** None. **S. Bremser:** None. **P. Kloppenburg:** None.

Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 590.23/G5

Topic: B.09. Intrinsic Membrane Properties

Title: Histone deacetylase inhibitor improved the dysfunction of hippocampal gamma oscillation and fast spiking interneurons in Alzheimer's disease model mice

Authors: *K. TAKASU, K. NIIDOME, M. HASEGAWA, K. OGAWA;
Pain & Neuroscience, Shionogi Co., Ltd., Toyonaka, Osaka, Japan

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

impairment in Alzheimer's disease (AD). Recent studies suggest that post-translational modification of histone protein via acetylation is a fundamental molecular mechanism for regulation of synaptic plasticity and memory formation. However, little is known about roles for histone acetylation in the hippocampal gamma oscillation. In this study, we investigated whether histone acetylation regulate kainate-induced gamma oscillation and its important regulator, fast-spiking interneurons, by using acute hippocampal slices of AD model mice (PSAPP transgenic mice). We found that deficits of kainate-induced gamma oscillation in PSAPP mice, and application of Donepezil, a clinically used drug for AD treatment, recovered the gamma oscillation. We demonstrated for the first time that histone deacetylase (HDAC) inhibitor suberoylanilide hydroxamic acid (SAHA) elevated the gamma oscillation level, and the effect of SAHA was accompanied with the elevation of histone H3 and H4 acetylation. The effects of SAHA on the gamma oscillation and histone acetylation were abolished by co-application of histone acetyltransferase (HAT) inhibitor C646, showing that histone acetylation is a key mechanism for regulation of gamma oscillation by SAHA. To further investigate the mechanisms underlying the reduction of gamma oscillation in PSAPP mice and its rescue following treatment of SAHA, we evaluated function of fast spiking interneurons, which were reported to mainly regulate gamma oscillation. We found impairments of both synapse-dependent and independent activity of fast spiking interneurons in PSAPP mice; the former being depolarized resting membrane potentials and increased spontaneous firing frequency and the latter being impaired intrinsic and kainate-induced excitability. Interestingly, SAHA and donepezil, both which improved reduction of gamma oscillation in PSAPP mice, differently rescued impairments in fast spiking interneurons. SAHA rescued both synapse-dependent and independent dysfunction by restoring spontaneous firing activity and intrinsic and kainate-induced excitability of fast spiking interneurons. By contrast, donepezil rescued only synapse-dependent dysfunction by restoring spontaneous firing activity. These results have indicated a novel mechanism that HDAC inhibition improved impairment of gamma oscillation in PSAPP mice by restoring synapse-dependent and independent dysfunction of fast spiking interneuron as pathological mechanism of AD.

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

Poster

590. Modulation of Neuronal Firing Properties I

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Program#/Poster#: 590.24/G6

Topic: B.09. Intrinsic Membrane Properties

Support: P01 NS079419

Title: Maintaining neuronal properties during growth with local and global homeostatic regulation

Authors: *H. GURNANI[1], T. O'LEARY[2], E. MARDER[3];
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Abstract: Neurons in the pacemaker circuit of the crustacean pyloric rhythm can maintain similar bursting rhythms despite morphological growth (Bucher et al 2005). However, intrinsic and synaptic properties, membrane resistance and capacitance, as well as calcium dynamics change dramatically as neurons grow and dendritic trees elaborate. How can neurons maintain ongoing activity in spite of these perturbations? Using computational modelling, we show that biologically plausible activity-dependent feedback can maintain bursting rhythms in growing neurons. We first explored a global regulation model in which calcium homeostasis is maintained through activity-dependent plasticity of ionic conductances. Although the model maintained calcium homeostasis and generated steady state bursting rhythms, different-sized single compartment geometries resulted in variable burst period and duty cycle. We then modified the regulation scheme by introducing local activity-dependent protein translation and degradation in dendritic compartment, along with global transcriptional regulation at the soma. This allowed geometries of varying surface area develop steady state bursting rhythms with similar burst period and duty cycle. Interestingly, geometries beyond a critical neuronal size were able to maintain steady state bursting, suggesting an asymmetry in the emergence of activity homeostasis with varying neuronal size. To further test of the regulation model, we implemented it in large-scale, morphologically realistic, reconstructed Stomatogastric Ganglion neuron models and modeled neuron growth. We first constructed morphologies at intermediate stages of branching by removing higher order dendrites. We observed that morphologies beyond a certain stage of branching developed similar steady state bursting under local regulation of different conductances, despite significant increase in dendritic area. Finally, in a model of continuous isometric growth, the rhythm stabilized when the morphologies reached 30-40% of their full size and the burst period was maintained through constant evolution of the conductances while the morphology continued to grow. These results show how the development and maintenance of activity patterns during neuronal growth can be achieved by a combination of global and local activity-dependent processes and indicate the existence of critical phases of growth where stable activity emerges.

Disclosures: **H. Gurnani:** None. **T. O'Leary:** None. **E. Marder:** None.

Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 590.25/G7

Topic: B.09. Intrinsic Membrane Properties

Support: Joint Research Program between JSPS and CNRS

Title: Ectopic spike firings of the hippocampal mossy fibers by application of kainate to the distal axons

Authors: *H. KAMIYA[1], C. MULLE[2];
[1]Hokkaido Univ. Grad. Sch. of Med., Sapporo, Hokkaido, Japan; [2]Interdisciplinary Inst. for Neuroscience, CNRS UMR 5297, Univ. of Bordeaux, Bordeaux, France

Abstract: Spike initiation at the proximal part of the axon, usually at the axon initial segment (AIS), is supported by the high excitability due to dense expression of voltage-gated sodium channels in the proximal axons. Robust directional propagation of axonal spikes from AIS to the distal axons and the terminals are well preserved for orthodromic conduction in the physiological conditions. However, in particular pathological conditions such as epilepsy, it has been suggested that ectopic spikes are generated at the distal axons and propagate antidromically to the soma. In this study, loose patch clamp recordings of axonal spikes from single mossy fiber boutons, in combination with local perfusion around the recording sites, were performed in mouse hippocampal slice preparations. Ca^{2+} free perfusing solution in the bath was used for minimizing indirect actions due to neurotransmitters released from surrounding cells. Hippocampal mossy fiber boutons were identified under observation with IR-DIC optics with large size of 4-8 μm in diameter and localization in the stratum lucidum, and were confirmed by the similar appearance of fluorescent labelled structures in Thy1-GFP transgenic mice. Mossy fiber boutons elicited spikes in all-or-none fashion following stimulation to the granule cell layer in the dentate gyrus. Under resting conditions, few spontaneous firings of axonal spikes were observed in most recordings from single mossy fiber boutons. Upon switching of the local perfusing solution to that containing low concentration of kainate at 0.2 μM, sporadic axonal spikes appeared immediately in most cases, possibly reflecting ectopic spontaneous discharges at distal axons by depolarization due to application of kainate. In some recordings, bursts of axonal spikes appeared and outlasted the period of kainate application for a prolonged time up to 5-10 min, similar to barrage firings observed in some neurons after strong neuronal activities. These results suggest the existence of cellular mechanisms leading to plastic changes in excitability of the distal axons of hippocampal mossy fibers by activation of kainate receptors.

Disclosures: H. Kamiya: None. **C. Mulle:** None.

Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 590.26/G8

Topic: B.09. Intrinsic Membrane Properties

Title: Effect of axotomy injury on the membrane elasticity of neurons

Authors: *S. BAY, B. POLAT, G. OZTURK;
Regenerative and Restorative Med. Res. Ctr. (REMER), Istanbul Medipol Univ., Istanbul - TURKEY, Turkey

Abstract: Plasma membrane stiffness/elasticity/tension is defined as the total force needed to deform the plasma membrane and has two components; the in plane tension which is the actual stiffness of the lipid bilayer. And the cortical tension which is due to membrane to cortex attachments and is derived from the cytoskeletal meshwork attached to the plasma membrane at the cell cortex. Evidence shows that numerous biological processes like vesicle trafficking and cell motility, is due to the active interplay of these forces and the cell stiffness actually play role in control of cell polarization and shape maintenance.
Among a lot of techniques for measuring the membrane tension, optical tweezers provide the most direct measurement by pulling an optically trapped bead and extracting a force value from the displacement of the bead. There are numerous studies about the effect of the extracellular substrate to neuron structure, morphology and axonal features, yet there are no studies. In this study, we aim to explore the changes in neural membrane tension after axonal damage.
In accordance with this purpose, dorsal root ganglia neurons were enzymaticly and mechanically dissociated. Neurons were sorted via density gradient centrifugation and cultured on laminin coated glass bottom petri dishes. The surface of 3 μm diameter polystrene beads were positively charged via an overnight poly-l-lysine coating protocol and beads were then tethered to neurons with an optical tweezer at 100X magnification. Laser axotomy was performed, the bead was pulled perpendicular to the cortical forces. Displacement was measured and forces were calculated.
In high percentage of axotomised neurons (60%) cortical forces increased upon axotomy. With help of newly avaliable technology and a well constructed experimental paradigm we were able to gather data about neural cell membrane machanics. Possible implications and applications of this increase will be discussed.

Disclosures: S. Bay: None. **B. Polat:** None. **G. Ozturk:** None.

Poster

590. Modulation of Neuronal Firing Properties I

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Topic: B.09. Intrinsic Membrane Properties

Support: We thank H. Wratil for outstanding technical assistance.

TR-SFB134 to PK

CONNECT to PK

Title: Electrophysiological characterization of paraventricular nucleus neurons

Authors: *A. C. KLEIN[1,2], P. KLOPPENBURG[1,2];
[1]Biocenter, Inst. For Zoology, Cologne, Germany; [2]Cologne Excellence Cluster on Cell. Stress Responses in Aging-Associated Dis. (CECAD), Univ. of Cologne, Cologne, Germany

Abstract: The Paraventricular Nucleus of the Hypothalamus (PVN) is an important autonomic control center in the brain that contains a heterogeneous neuron population. It plays an important role in regulating autonomic renal and cardiovascular functions, stress responses, and is also crucial for controlling the energy balance. For instance, lesioning the PVN causes hyperphagic obesity in rats (Physiol & Behav 1981; 27(6): 1031-1040) and injection of the melanocortin-4 receptor agonist melanotan-2 into the PVN reduces food intake, while the antagonist agouti-related-protein (and neuropeptide Y) increases food intake (Neuron 1999; 24: 155-163). These results demonstrate, that the PVN is an important integration sites in the hypothalamus for both neuroendocrine and autonomous pathways.
Within the PVN three different types of neurons have previously been identified in rats: Magnocellular neuroendocrine neurons (MC), parvocellular neurosecretory (NS) and preautonomic (PA) neurons (J Physiol 1991; 434, 271-93). These neuron types can be identified by a number of anatomical features, molecular markers, and intrinsic electrophysiological properties. While the cell bodies of MC neurons are relatively big and mainly located in the medial part of the PVN, cell bodies of parvocellular NS and PA neurons are smaller and predominantly located in the anterior and posterior PVN respectively. Furthermore, MC and parvocellular NS neurons extend their projections primarily to the median eminence where they regulate pituitary function, whereas PA neurons mainly project to hindbrain nuclei and the spinal cord. In addition, these neurons differentially express a variety of peptides such as oxytocin, vasopressin, corticotropin-releasing factor, or thyrotropin-releasing hormone (J Comp Neurol 2009; 10(5), 423-41). In rats, PVN neurons can also be identified by their distinct electrophysiological properties (J Physiol 1991; 434, 271-93).
Here we provide a comprehensive electrophysiological characterization of the three PVN neuron

types in mice to establish a solid base for future experiments that aim to investigate the modulation of the PVN network under physiological and pathophysiological contexts. By performing perforated patch-clamp recordings in hypothalamic mouse brain slices we found that the distinct neuron types can be identified by specific electrophysiological characteristics such as delayed action potential onset after hyperpolarization in the case of MC neurons, generation of low threshold spikes in parvocellular PA neurons, and typical spike frequency adaptation behavior in parvocellular NS neurons.

Disclosures: A.C. Klein: None. **P. Kloppenburg:** None.

Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

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Program#/Poster#: 590.28/G10

Topic: B.09. Intrinsic Membrane Properties

Title: Characterization of ion channel conductance and mRNA expression levels in identified neurons of a parthenogenetic crayfish

Authors: *C. STAEDELE, A. G. VIDAL-GADEA, W. STEIN;
Sch. of Biol. Sci., Illinois State Univ., Normal, IL

Abstract: The activity of individual neurons is achieved through the combined action of ion channels in the cell membrane. However, the ion channel conductance levels vary between animals of the same species, and between neurons of the same type (Schulz et al. 2006; Golowasch et al. 1999, 2002; Liss 2001), indicating that this variability may be an important factor in maintaining robust neural function over time. While all neurons have mechanisms that homeostatically regulate intrinsic excitability and responses to synaptic input, it is unclear whether differences between animals are due to genetic variability between individuals or to experience-dependent homeostatic plasticity. Addressing this question has been challenging in existing animal models due to lack of identified neurons and circuits, weak access to cellular and circuit dynamics, or the availability of genetic tools. We are studying conductance level variability using single-cell electrophysiology and molecular tools in identified neurons of the marbled crayfish, *Procambarus fallax forma virginalis*. Marbled crayfish are an all-female species with genetically homogenous animals that reproduce parthenogenetically. To determine conductance diversity, we are measuring ion conductance levels in the same identified neurons of the stomatogastric ganglion and compare them across animals. In addition, we are measuring ion channel expression levels using single-cell qRT-PCR to confirm the results of our electrophysiological measurements. Previous studies in this system were carried out in wild-

caught crustaceans, and revealed a large variability of ion channel conductances levels and correlated mRNA levels (Schulz et al. 2006, 2007). We hypothesize that the variability between identified neurons is reduced in marbled crayfish as a result of their genetic homogeneity. To test this, we are raising animals under identical environmental conditions, minimizing differences in life-history, before conductance and expression levels are compared. A second test group consists of animals raised at different environmental conditions, for which we predict distinct conductance and expression levels in comparison to control as a result of homeostatic feedback mechanisms. We are also considering the possibility to manipulate gene expression using transgenic animals by introducing genetic constructs into the germline of marbled crayfish.

Disclosures: C. Staedele: None. **A.G. Vidal-Gadea:** None. **W. Stein:** None.

Poster

590. Modulation of Neuronal Firing Properties I

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Program#/Poster#: 590.29/G11

Topic: B.09. Intrinsic Membrane Properties

Support: PNII-RU-TE-2014-4-0406/2015 contract no. 169/2015

Title: Effects of periodic stimulation on cortical circuits as a function of stimulated population properties

Authors: *A. DABACAN, V. V. MOCA, R. C. MURESAN;
Exptl. and Theoretical Neurosci., Romanian Inst. of Sci. and Technol., Cluj Napoca, Romania

Abstract: Novel experimental techniques, such as optogenetics, aim to dissect network function, acting on subsets of cells with various physiological and functional properties. The study of oscillatory mechanisms involved in information processing greatly benefits from these tools. In particular, it is important to understand the differential involvement of neuron subtypes in the entrainment and sustainment of robust cortical oscillations.
We used models of cortical microcircuits to test network frequency-response as a function of stimulated population (excitatory: E or inhibitory: I). Excitatory neurons were modeled as pure integrate-and-fire (IF) neurons, while interneurons were either IF or Izhikevich neurons exhibiting membrane resonance (RES). By switching interneuron model, we obtained two network types: IF-IF and IF-RES.
When either E or both E and I neurons were periodically stimulated, we found circuit resonance for all networks. At low stimulation frequencies (< 15 Hz), both IF-IF and IF-RES networks sustained oscillations, with a frequency in the range of 18-30 Hz, irrespective of the input. For

stimulation frequencies in the range of 18–40 Hz, networks were locked to input frequencies, while at higher stimulation frequencies, oscillation stabilized within the 18-30 Hz range, frequency was higher for the IF-RES network and power was higher for IF-IF networks. The latter exhibited circuit resonance peaks at multiples of main peak frequency when only E neurons were stimulated, indicating an m-to-n locking phenomenon. This was not the case for IF-RES networks, where interneuron resonance constrained the circuit resonance to a limited domain. When input was delivered to I cells only, IF-IF networks were not able to sustain oscillations, while IF-RES nets exhibited a circuit resonance peak matching interneuron resonance frequency. Our results indicate complex network responses to periodic stimulation, dependent on input populations and their properties, with important implications for optogenetic studies of cortical oscillations.

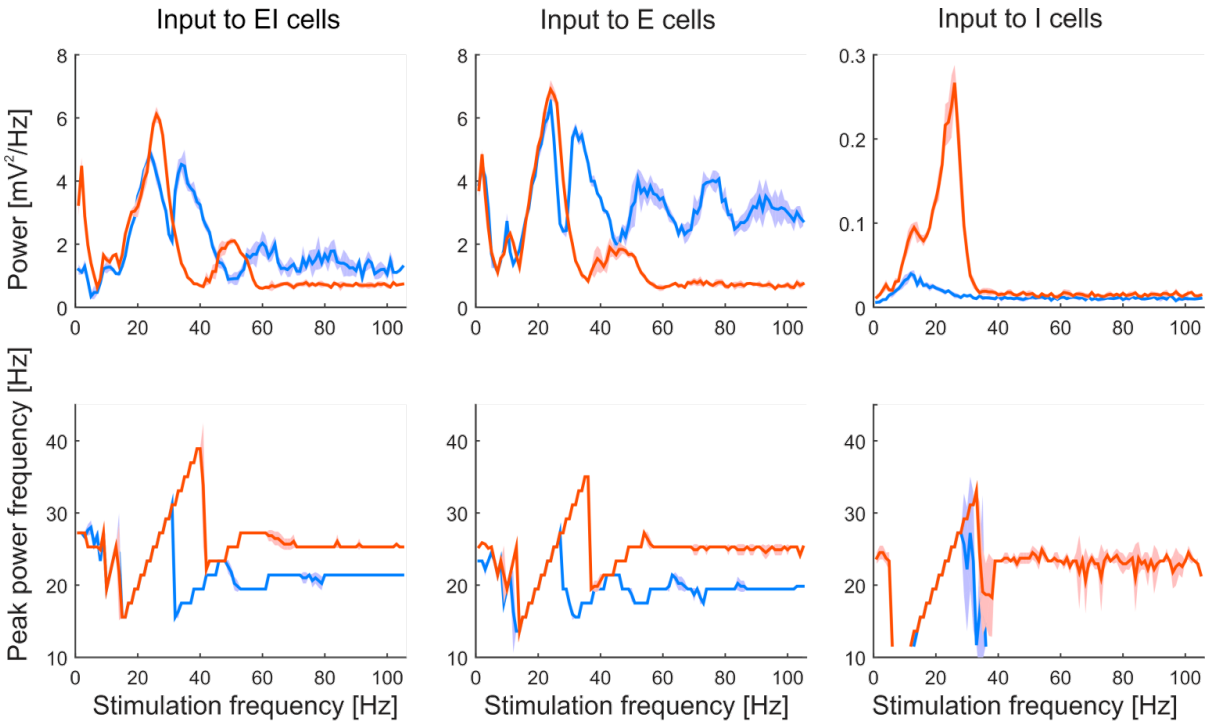

***Figure 1*. Network response to periodic stimulation**: **Blue**: IF-IF networks; **Orange**: IF-RES networks; **Top**: maximum power vs. stimulation frequency; **Bottom**: frequency of maximum power vs. stimulation frequency; **Left**: Stimulation delivered to E and I cells; **Middle**: Stimulation delivered to E cells only; **Right**: Stimulation delivered to I cells only; Mean and standard deviation are computed across 5 randomly generated networks.

Disclosures: A. Dabacan: None. **V.V. Moca:** None. **R.C. Muresan:** None.

Poster

590. Modulation of Neuronal Firing Properties I

Location: Halls B-H

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Program#/Poster#: 590.30/G12

Topic: B.09. Intrinsic Membrane Properties

Support: Wellcome Trust

Title: Using genetically encoded voltage sensors to measure voltage propagation along neuronal cables

Authors: *M. RIGBY[1], J. BURRONE[2];
[1]Ctr. for Developmental Neurobio., [2]King's Col. London, London, United Kingdom

Abstract: The propagation of voltage along neuronal cables depends on the resting and active properties of the membrane, as well as the intracellular resistivity. Although many studies have characterized the distribution of voltage-gated ion channels that contribute to the steady-state and active conductances of a neurite, much less is known about how localized changes in axial resistance influence voltage attenuation. Here we used the genetically-encoded voltage indicators ASAP-1 and ACE-2N-4AA to simultaneously record the membrane voltage at many locations along the dendritic and axonal arbors of dissociated neurons. Negative current steps were injected at the soma using a patch pipette, and the passive propagation of the hyperpolarizing pulse imaged along the neurites. By correlating the length constant of the voltage decay to structural features of the sub-compartments we highlight how variability in passive neuronal properties can modulate slow changes in membrane potential. These findings have important implications for how neurons integrate synaptic inputs and regulate the action potential. By imaging at much higher frequencies we next aim to examine how such fast changes in membrane potential are affected by local differences in steady-state conductances and intracellular resistivity

Disclosures: **M. Rigby:** None. **J. Burrone:** None.

Poster

591. Oscillations and Synchrony: Other III

Location: Halls B-H

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Program#/Poster#: 591.01/G13

Topic: B.10. Network Interactions

Support: Wellcome Trust

Title: Duration-dependent online effects of transcranial alternating current stimulation (tACS)

Authors: ***M. NOWAK**[1], E. HINSON[1], A. GUERRA[2], A. POGOSYAN[1], F. VAN EDE[1], A. QUINN[1], P. BROWN[1], C. J. STAGG[1];
[1]Univ. of Oxford, Oxford, United Kingdom; [2]Univ. Campus Bio-Medico, Rome, Italy

Abstract: Transcranial alternating current stimulation (tACS) is a non-invasive technique capable of interacting with endogenous brain oscillations in a frequency-specific manner, which offers a unique opportunity to study the causal role of brain rhythms in behaviour and how they relate to dysfunction, thus paving the way to new therapeutic applications in disorders associated with abnormal neuronal oscillations, such as Parkinson's disease. However, current understanding of the mechanisms that underlie the effects of tACS is limited, which in turn hampers the rational optimization of human experiments.
Recent work on tACS for the first time has provided evidence that oscillations at beta (15–30 Hz) and gamma (30–90 Hz) frequency range are causal, rather than epiphenomenal, to motor behaviour. The aim of the present study was to investigate the neurophysiological effects of beta- and gamma-frequency tACS in healthy subjects (n=20). To this end, we employed TMS to evaluate the effects of tACS on the following: (1) the global corticospinal excitability, as indexed by motor evoked potentials (MEPs), elicited by single-pulse TMS; (2) short interval intracortical inhibition (SICI) and (3) intracortical facilitation (ICF) in the left primary motor cortex (M1) following paired-pulse TMS, considered to reflect $GABA_A$-mediated synaptic inhibition and glutamatergic facilitation, respectively. tACS was applied over the left M1 and the contralateral orbit for 20 min at intensity adjusted below individual phosphene- and discomfort threshold. All TMS protocols were performed at rest before, during (at 5 and 15 min) and after tACS. A single MEP and SICI were also recorded during movement preparation before and after tACS.
We found that high gamma tACS affected a single MEP and SICI in a differential manner during the stimulation. Specifically, SICI was decreased at an early stimulation period (5 min) but increased during a later period (15 min) as compared to the sham group. The opposite effect was observed for a single pulse TMS protocol. Moreover, we found that the observed responses at an early stimulation period proved to be a good predictor for individual responses measured during a later period. Finally, we found no after-effects of tACS. These experimental data provide evidence in support of differential duration-dependent online effects of tACS, which resemble

homeostatic-like plasticity, although no effect lasting beyond the stimulation period was observed. In addition, the data demonstrate the predictive power of initial responses to tACS, which could be helpful in screening responsive subjects or probing stimulation efficacy before therapeutic application.

Disclosures: **M. Nowak:** None. **E. Hinson:** None. **A. Guerra:** None. **A. Pogosyan:** None. **F. van Ede:** None. **A. Quinn:** None. **P. Brown:** None. **C.J. Stagg:** None.

Poster

591. Oscillations and Synchrony: Other III

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Support: University of Michigan Rackham Conference Travel Grant

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Young Investigator Award from the Brain and Behavioral Research Foundation

Alfred P. Sloan Foundation Fellowship

Title: Oscillations contribute to memory consolidation by changing criticality and stability in the brain

Authors: ***J. WU**[1], Q. M. SKILLING[2], N. OGNJANOVSKI[3], S. J. ATON[3], M. ZOCHOWSKI[2]; [1]Applied Physics Program, [2]Biophysics Program, [3]Dept. of Molecular, Cellular, and Developmental Biol., Univ. of Michigan, Ann Arbor, MI

Abstract: Oscillations are a near universal feature of every level of brain dynamics and have been shown to contribute to many functions, such as neural binding, information encoding and processing, and subsystem integration. Despite almost a century of active research and many proposed hypotheses, the role of oscillations in shaping network dynamics is still not fully understood. To investigate the fundamental mechanism underpinning oscillatory activity, the properties of heterogeneous networks are compared in situations with and without oscillations, both computationally and experimentally. Our results show that both network criticality and stability are changed in the presence of oscillations. Criticality describes the network state of neuronal avalanche, a cascade of bursts of action potential firing in neural network. The

branching parameter σ is defined as the average number of subsequent active neurons at the next time point triggered by one neuron. σ<1, σ=1, σ>1 correspond to subcrtitical, critical and supercritical state respectively. Preliminary results indicate that an increase of σ is associated with better learning performance. Stability measures how stable the spike timing relationship between neuron pairs is over time. Using a detailed spiking model, we found that the branching parameter σ changes relative to oscillation and structural network properties, corresponding to transmission among differnt critical states. Also, analysis of functional network structures shows that the oscillation helps to stabilize neuronal representation of memory. Further, quantitatively similar results are observed in biological data recorded in vivo. By inhibiting parvalbumin-expressing (PV+) interneurons, delta (0.5-4Hz), theta (4-12Hz) oscillations are blocked, leading to poor learning behavior. Previously, supracritical state is thought to be associtated with a high Excitatory/Inhibitory ratio. However, the change in branching parameter reflects that the system does not have to transit to supracriticality, even though the network becomes more excitatory caused by inhibition of PV+. In summary, we have observed that, by regulating the neuronal firing pattern, oscillations affect both criticality and stability properties of the network, and thus contribute to memory formation.

Disclosures: **J. Wu:** None. **Q.M. Skilling:** None. **N. Ognjanovski:** None. **S.J. Aton:** None. **M. Zochowski:** None.

Poster

591. Oscillations and Synchrony: Other III

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Program#/Poster#: 591.03/G15

Topic: B.10. Network Interactions

Support: OXION studentship Wellcome Trust

Title: Abnormal theta and gamma oscillations in mice lacking the GluA1 AMPAR subunit

Authors: ***A. BYGRAVE**[1], D. KULLMANN[2], D. BANNERMAN[1], D. KÄTZEL[1,3,2];
[1]Univ. of Oxford, Oxford, United Kingdom; [2]Univ. Col. London, London, United Kingdom; [3]Univ. of Ulm, Ulm, Germany

Abstract: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) mediate the majority of excitatory neurotransmission in the mammalian brain. AMPARs are tetramers, formed from GluA1-4 subunits. The GluA1 subunit is of particular interest, as AMPARs containing this subunit are involved in certain forms of synaptic plasticity. The lack of pharmacological agents to manipulate AMPARs with subunit specificity has led to the

generation of genetically modified mice which lack different AMPAR subunits. Mice lacking the GluA1 subunit (GluA1 KOs) lack specific forms of synaptic plasticity, but maintain intact spatial reference memory. In contrast, spatial working memory (SWM), and other forms of short term memory, is abolished in GluA1 KO mice. In addition, GluA1 KO mice show profound novelty induced hyperactivity. The in vivo electrophysiological phenotype of GluA1 KO mice has been studied less. Local field potential (LFP) oscillations in the theta (4-12 Hz) and gamma (30-80 Hz) frequency range are thought to be important for memory processes including SWM. We have recorded LFPs from the hippocampus and prefrontal cortex of awake, behaving, GluA1 KO and wildtype control mice. During exploration of a novel environment the relative theta power in GluA1 KO mice is increased, and the peak frequency of the oscillation is reduced in the dorsal hippocampus compared to control. Under the same conditions the relative gamma power in the dorsal hippocampus is reduced in GluA1 KO mice compared to controls. Furthermore, we engineered a virus to express GluA1, to test if it was possible to rescue these oscillatory deficits by reintroducing GluA1 into the hippocampus of adult GluA1 KO mice. Injection of this rescue virus into the hippocampus led to expression of GluA1, particularly in the CA3 region. The rescue virus was able to normalise the relative theta power, but not the relative gamma power in the dorsal hippocampus. Reintroduction of GluA1 also partially rescued novelty induced hyperactivity in GluA1 KO mice. Our results suggest that GluA1 is required for normal theta and gamma oscillations, and indicate that a loss of GluA1 from the hippocampus could, at least in part, underlie the novelty induced hyperactivity seen in GluA1 KO mice.

Disclosures: **A. Bygrave:** None. **D. Kullmann:** None. **D. Bannerman:** None. **D. Kätzel:** None.

Poster

591. Oscillations and Synchrony: Other III

Location: Halls B-H

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Program#/Poster#: 591.04/G16

Topic: B.10. Network Interactions

Support: KAKENHI (no. 26880019)

ONR-MURI grant N000141010278

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Title: Modeling spike synchrony generated by modulatory common input through nmda-type synapses

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Abstract: Common excitatory input to neurons increases their firing rates and the strength of the spike correlation (synchrony) between them. Little is known, however, about the synchronizing effects of modulatory common input. Here we show that modulatory common input with the slow synaptic kinetics of NMDA receptors enhances firing rates and also produces synchrony. Tight synchrony (correlations on the order of milliseconds; Amarasingham et al, J. Neurophysiol. 107:517, 2012) always increases with modulatory strength. Unexpectedly, the relationship between strength of modulation and strength of loose synchrony (tens of milliseconds; Dong et al, J. Vis. 8: 1, 2008) is not monotonic: The strongest loose synchrony is obtained for intermediate modulatory amplitudes. This finding explains recent neurophysiological results showing that in cortical areas V1 and V2, presumed modulatory top-down input due to contour grouping increases (loose and tight) synchrony, but that additional modulatory input due to top-down attention does *not* change tight synchrony, and actually *decreases* loose synchrony (Martin and von der Heydt, J. Neurosci 35:6860, 2015). These neurophysiological findings are understood from our model of integrate-and-fire neurons under the assumption that contour grouping as well as attention lead to additive modulatory common input through NMDA-type synapses. In contrast, circuits with common projections through model AMPA receptors did not exhibit the paradoxical decrease of synchrony with increased input. Our results suggest that NMDA receptors play a critical role in top-down response modulation in visual cortex.

Disclosures: N. Wagatsuma: None. **R. von der Heydt:** None. **E. Niebur:** None.

Poster

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Topic: B.10. Network Interactions

Support: NRF-2014R1A2A1A0128

Title: Influence of whole-body vibration exercise on cortical activity in chronic stroke patients: functional near-infrared spectroscopy study

Authors: *J. JUNG[1], D.-S. CHOI[1], A. LEE[2], E. PARK[1], H.-G. KIM[2], H.-J. LEE[1], S. LEE[1], W. CHANG[1], Y.-H. KIM[1];

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Abstract: Introduction: Whole-body vibration (WBV) exercise can provide a proper somatosensory stimulation in stroke patients to promote functional improvement. However, there was a lack of study to investigate the neuroplasticity according to WBV exercise in stroke patients. The purpose of this study was to investigate the effects of whole-body vibration (WBV) exercise on cortical activity in patients with chronic stroke. **Methods:** Eighteen chronic stroke patients were randomly assigned to either the WBV group (n = 12) or control group (n = 6). The WBV group received vertical whole-body vibration (Galileo Advanced plus, Novotec Medical, Germany**)** with intensity of the 20 Hz and the amplitude of 4 mm for 5 minutes with half-squat position on the platform. Cortical activation was measured before and after stimulation period by relative changes of oxygenated hemoglobin (oxyHb) in primary motor cortex (M1), premotor cortex (PM), supplementary motor area (SMA), somatosensory cortex (S1), and prefrontal cortex (PFC) using a functional near infrared spectroscopy (NIRScout, NIRx, Germany). Two-way repeated measures ANOVA was used for statistical analysis. **Results:** OxyHb concentration for M1 and S1 of the unaffected side was significantly increased after vibratory stimulation in the WBV group, but not in the control group. There were also significant interaction effects in group and time for M1. OxyHb concentration for PFC of the affected hemisphere and SMA of the unaffected side was significantly higher in the WBV group than the control group. There were significant main effects between groups in the affected PFC and the unaffected SMA. **Conclusion:** This study provided the evidence that WBV exercise have positive influences on cortical activity of motor network and prefrontal area in the chronic stroke patients (Supported by the NRF grant funded by the Korea government (NRF-2014R1A2A1A0128)).

Disclosures: J. Jung: 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; Supported by the NRF grant funded by the Korea government. **D. Choi:** 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; Supported by the NRF grant funded by the Korea government. **A. Lee:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Supported by the NRF grant funded by the Korea government. **E. Park:** 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; Supported by the NRF grant funded by the Korea government. **H. Kim:** 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; Supported by the NRF grant funded by the Korea government. **H. Lee:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research

relationship even if those funds come to an institution; Supported by the NRF grant funded by the Korea government. **S. Lee:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Supported by the NRF grant funded by the Korea government. **W. Chang:** 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; Supported by the NRF grant funded by the Korea government. **Y. Kim:** 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; Supported by the NRF grant funded by the Korea government.

Poster

591. Oscillations and Synchrony: Other III

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BFU2014-52467-R

FLAGERA-PCIN-2015-162-C02-01

MINECO (Spain)

WaveScalES, EC FET Flagship HBP SGA1 720270

Title: Metastable dynamics underlying the multiscale organization of slow oscillations

Authors: *M. MATTIA[1], M. PEREZ-ZABALZA[2], N. TORT-COLET[2], M. V. SANCHEZ-VIVES[2,3];
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Abstract: Slow oscillations (SO) are a stereotyped activity pattern pervasively expressed during slow-wave sleep and deep anesthesia by the cerebral cortex of many species. SO in sensorial cortices are known to mirror early neuronal processing of environmental stimuli, and occur

simultaneously in cell assemblies at different cortical depths and positions as a concerted multiscale activity. In visual cortex (V1) for instance, such nonlinear network dynamics may contribute to sustain input-related activity once visual stimuli are removed or when the stimulation sequence is incomplete, a computational primitive allowing integration of information across time. The dynamic roots of such concerted activity is still an open issue which here we address by carrying out a detailed exploration of the multiscale dynamics of SO to understand the rules of columnar function. To this end, we recorded the synaptic and neuronal activity underlying SO from V1 of anesthetized rats (ketamine-medetomidine, n =18 adult male Wistar rats), by extracting from a 16-channel silicon probe both current source densities (CSDs) and MUAs across all cortical layers.Our first finding is that layer 5 (L5) assemblies give rise to hysteresis loops like in flip-flop computational units. This confirms the leading role of L5 in generating and sustaining the Up states of SO with the highest firing rate. Yet, it supports the long standing hypothesis that local cortical networks are capable to express attractor dynamics, also suggested to underlie cognitive functions like working memory and decision making. We demonstrate that this attractor dynamics are history-dependent and most likely mediated by synaptic reverberation and activity-dependent adaptation mechanisms. Columnar activation during SO display a stereotyped ascending pattern, reproducibly initiated in layer 6 (L6) spreading upward towards the cortical surface. From CSD analysis, this is due to an early synaptic input that L6 assemblies receive, inducing a time lag of 14 ± 11 ms between L6 and L5 Up onsets. By inactivating LGN by TTX injections, we demonstrate that the input to L6 is of cortical origin, likely due to travelling Up wavefronts. Although the thalamus had no apparent role in columnar activation, we found that it contributes to modulate the stability of Down states in infragranular layers. This evidence recasts the cortico-thalamo-cortical loop as also a modulator of SO in sensorial areas, strengthening the role of L6 as a fundamental hub of neuronal activity produced at macroscopic scale. A role expressed not only in response to sensory stimulation but also under self-sustained or spontaneous state like slow-wave activity.

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Poster

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Support: BFU2014-52467-R

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EU FP7 CORTICONIC (600806)

Title: Slow wave propagation in the cortical network and laminar excitability

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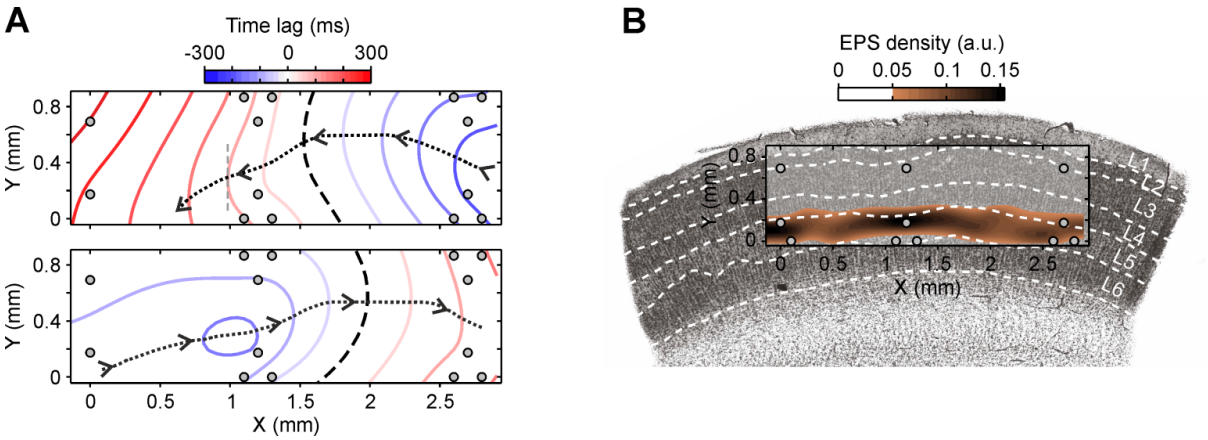
Abstract: Slow oscillations of neural activity spontaneously emerge and propagate across the cerebral cortex during deep sleep and anesthesia, an activity also expressed in brain slices and cortical slabs. The mechanistic origin of slow oscillations integrates the local excitability of cell assemblies and their mutual interaction. Here, we address this issue focusing on ongoing slow waves spontaneously generated in neocortical slices and recorded by means of a 2D array of 16 electrodes designed to probe the neuronal activity at different spatial scales. On top of the largely variable propagation modes observed, we reproducibly found a smooth strip of loci leading the slow wave fronts that overlapped with cortical layer 4 and 5. Along this strip, Up states were the longest and displayed the highest firing rate. Under control conditions of excitability and at a frequency of oscillations of 0.31 ± 0.12 Hz, the propagation mode was uncorrelated in time, highlighting a memoryless generation of slow waves. To model all these features, a multi-modular large-scale network of spiking neurons must incorporate a specific balance between local and intermodular connectivity. Modules must also work as relaxation oscillators with a weakly stable Down state and a peak of local excitability to model layer 4 and 5. These peculiar settings combined together provide an optimal sensitivity to the network structure and generate a richness of available propagation modes, a potential neuronal substrate for experience-driven flexibility.Figure legend. A. Two example slow-waves propagating in opposite directions across a cortical slice probed with a 16 electrode MEA (grey circles). Dotted lines with arrows indicate the early propagation strip (EPS) of the wavefronts. Consecutive (color coded) wavefronts are separated by 50 ms. B. EPS density and area covered by the MEA superimposed to the image of the corresponding cortical slice stained after electrophysiological recordings. Dashed lines, boundaries between layers. EPS density below 0.05 is not shown.



Disclosures: **M.V. Sanchez-Vives:** None. **C. Capone:** None. **B. Rebollo:** None. **P. del Giudice:** None. **M. Mattia:** None.

Poster

591. Oscillations and Synchrony: Other III

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Topic: B.10. Network Interactions

Support: BFU2014-52467-R

FLAGERA-PCIN-2015-162-C02-01

BFU2011-27094

Title: Non-synaptic wave propagation: modulation of slow oscillations by endogenous electric fields

Authors: ***B. REBOLLO**[1], S. GONZALEZ-ANDINO[2], R. GRAVE DE PERALTA MENENDEZ[2], M. SANCHEZ-VIVES[1,3];
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Abstract: The cerebral cortex is organized in circuits of neurons that interact via synaptic mechanisms. The synaptic activity co-exists with endogenous electric fields (EF) generated by neuronal activity. These changes in the electric environment of neurons due to the network activity have a feedback effect onto neurons (Fröhlich and McCormick Neuron, 67: 129. 2010; Anastassiou et al. Nature Neuroscience, 14: 217, 2011). To study the endogenous EF effect onto

the local network, we used an *in vitro* preparation of ferret visual cortex that generates slow oscillations (Sanchez-Vives and McCormick, Nature Neuroscience, 3: 1027, 2000), a pattern of activity typical of slow wave sleep and anesthesia. A complete vertical cut of the slice, perpendicular to the cortical surface, was performed, while the two sides of the cut remained in contact without discontinuity between them. This resulted into two independent networks on each side, each of which had its own oscillatory pattern of spontaneous UP states. The vertical cut allowed to isolate synaptic transmission from the EF propagation. We then characterized how the EF induced by slow wave oscillations propagated in the cortical tissue across the cut, therefore independent from synaptic transmission. Up states originated and propagated within one side of the slice, could be recorded across the cut albeit with strongly reduced amplitude, losing more than 50% of their original amplitude. Dampening was accompanied by an average delay of the propagated signal by 104 ms, resulting in a EF propagation speed of 14 mm/s, a value similar to the speed of wave propagation of slow oscillations under intact synaptic connections. The propagated EF had an impact on the activity generated across the cut. We explored this phenomena under different conditions, including spontaneous slow waves, electrical and chemically-induced responses and epileptiform discharges. Our results suggest that cortical patterns do not exclusively emerge from synaptically interconnected neurons but they are also shaped by the fields generated by the active network.

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Poster

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WaveScales, 604102 (Human Brain Project)

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Title: Modulation of cortical intrinsic bistability and complexity in the cortical network

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Abstract: The complexity of EEG-measured human cortical response evoked by transcranial magnetic stimulation can be estimated by the perturbational complexity index (PCI) (Casali et al. Science Translational Medicine, 5(198): 198ra105, 2013). PCI shows a significant correlation with the level of consciousness in human subjects across different conditions (sleep, anesthesia, coma), being thus valuable for diagnoses. Upon loss of consciousness, the complex pattern of causal interactions observed during wakefulness collapses into a stereotypical slow wave, suggesting that cortical bistability may play a role. Bistability is mainly expressed in the form of slow oscillations, a default pattern of activity that emerges from cortical networks in conditions of functional or anatomical disconnection. Here we aimed to investigate the cortical mechanisms underlying the regulation of complexity and causality in different brain states taking advantage of an in vitro preparation of the cerebral cortex that displays emergent slow oscillations (<1Hz). Recordings were obtained with a 16 channel multi-electrode array and network perturbation was induced by electric stimulation. The PCI algorithm used in humans was adapted to the local scale of the cortical slice. Different brain states were simulated by pharmacological manipulation. In particular, we investigated 3 network states: 1) spontaneous slow oscillations, 2) a state of increased network excitability through bath-applied kainate, and 3) an “awake-like” state, induced by means of mimicking the ascending activating systems by means of norepinephrine and carbachol. During slow oscillations we observed a response similar to that observed in non-REM sleep: an initial activation, followed by a silent state and break-off of deterministic activations. Under the effect of norepinephrine and carbachol instead, we observed a significant increase in PCI, correlated with prolonged deterministic effects similar to those corresponding to the awake state. These observations were not reproduced though by kainate. This in vitro results give us hints into the cortical mechanisms regulating complexity and causality, highlighting the central role of the intrinsic bistability typical of states of unconsciousness and demonstrating that complexity can be modulated pharmacologically.

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Poster

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Title: Cortical population dynamics during spindles and at the transition from NREM sleep

Authors: *D. LEVENSTEIN[1,2], B. O. WATSON[2,3], J. RINZEL[1], G. BUZSÁKI[2];
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Abstract: Neural activity during sleep is highly choreographed over multiple time scales, from global brain states at the scale of minutes to oscillatory events at the scale of seconds and neuronal assemblies at the scale of 10-100ms. Previously, we found that the sleep process shows distinct effects on the activity of high and low firing rate cortical neurons. These effects are sleep-state-specific and can be distinguished by their correlation with the incidence of different sleep oscillations. To this end, we sought to further characterize how the state-dependent choreography of sleep oscillations organizes the activity of specific neuronal populations. From in vivo multi-site recordings in frontal cortices of naturally sleeping rats, we find that the transition out of NREM sleep is characterized by increased power in a broad range of intermediate frequencies (8-30Hz, “sigma” and “beta" bands of the LFP) and an increased incidence of thalamocortical spindles. While this transitional state has been previously described in the rodent as intermediate sleep for the transition from NREM to REM, we find that it is also seen before brief NREM “microarousals”, as well as rare transitions directly from NREM to wakefulness. However, the transition from NREM to REM uniquely shows coherent activity in these frequency ranges between the cortex and hippocampus and is associated with increased activity of cortical low firing rate neurons. We find that the population activity of putative inhibitory neurons shows strong spindle-band fluctuations during this transitional state and that a subpopulation of inhibitory neurons are tightly rate- and phase-coupled to the thalmocortical spindle oscillation. These spindle-locked interneurons are activated early in the spindle and burst with reliable spindle-cycle inter-burst intervals. Paralleling this population, we find another subpopulation of spindle phase-coupled putative inhibitory units which are inactivated at the beginning of the spindle and gradually increase in firing rate over the course of the spindle. While we see counterparts to these inhibitory populations in the high firing rate excitatory populations, the more striking finding is that spindles act to segregate high and low firing rate putative pyramidal units, which show highly correlated firing within their own rate groups but anti-correlated activity with each other. These results suggest that one function of thalamocortical sleep spindles is to temporally segregate specific cortical populations and that the transition out of NREM is a unique time in sleep during which this can happen.

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Poster

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MH107396

NS90583

MH54671

Title: Functional properties of distinct excitatory cell types in the hippocampal dentate gyrus

Authors: *Y. SENZAI[1], L. ROUX[2], E. STARK[3], G. BUZSAKI[2];
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Abstract: The dentate gyrus (DG) in the hippocampus has two types of excitatory cells: granule cells, which are the main output cells from DG to CA3, and mossy cells, which reside in the hilus proper and provide recurrent connections to granule cells. To understand computation in the DG circuitry, it is important to unequivocally identify granule cells and mossy cells in terms of physiological characteristics. However, there is only a limited amount of knowledge regarding these issues to date. To address these questions, we performed large-scale recordings of local field potential (LFP) and unit firing from DG of freely moving mice combined with physiological and optogenetic identification of each cell type. For physiological identification, we used several parameters, including the spike width of the averaged waveform, the burst index based on the auto-correlogram, the ratio of the firing rates during awake to during slow wave sleep (SWS), and the amplitude of the dentate spikes types 2 (DS2) observed during SWS. By taking the DS2 amplitude at the exact recording site each unit was recorded, we estimated the anatomical location of each unit. Based on these parameters, we found two clusters of putative excitatory cells: one closer to the molecular layer and another closer to the hilus of DG. We detected putative monosynaptic connections between these units based on unit-unit cross-correlogram and found the significant spike transmission with 2-3 ms delay from units in the cluster closer to the molecular layer to units in the cluster closer to the hilus but not the other way. Considering the known strong synaptic connections from granule to mossy cells, we conclude that the two clusters correspond to granule cells and mossy cells, respectively. Optogenetic tagging using Drd2-Cre mice, which specifically label mossy cells in DG, confirmed that the hilus cluster corresponds to mossy cells. Based on this classification, we then

characterized the physiological properties of granule cells and mossy cells. We found that granule cells had lower firing rates compared to mossy cells, especially during awake periods . We also found that granule cells' firing rate is more strongly modulated by gamma oscillation and DS2 during SWS. Next, we characterized the spatial information coding by each cell type on a linear or a figure eight maze. Mossy cells had multiple place fields and higher peak firing rates, whereas granule cells typically had a single place field and lower firing rates. In summary, our results revealed the important differences between granule cells and mossy cells in terms of physiological characteristics and spatial information coding in behaving and sleeping mice.

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Poster

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Topic: B.10. Network Interactions

Support: MH107662

MH107396

NS90583

MH54671

Title: Neurogrid: high-frequency hippocampal-neocortical coordination

Authors: *D. KHODAGHOLY, J. N. GELINAS, G. BUZSAKI;
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Abstract: Large-scale recording from neural networks and their interactions are critical for understanding how information is processed and transmitted in the brain. Coupling between specific limbic and neocortical brain regions is implicated in aspects of memory acquisition, consolidation, and retrieval, but how these interactions are coordinated over topographically diverse functional networks during online task performance and in offline states is unclear. We designed and developed a large-scale array to cover most of the dorsal surface of rat cortex, allowing simultaneous recording from multiple cortical areas and improved localization of activity in higher order association cortices. The array consists of 120-384 electrodes arranged in clusters of 4 microelectrodes (20μm) for the purpose of spike recording and clustering. These clusters are spaced 200μm apart to cover an area shaped to match the dorsal cortical surface.Here

we examine large-scale hippocampal-neocortical interactions by simultaneous recording of local field potential (LFP) and spiking activity from a large part of the dorsal neocortical surface, from bregma to lambda, and hippocampus in freely moving rats. We identified a spatially confined, prominent high frequency oscillation (100-150 Hz) in posterior parietal cortex (PPC) that occurs mainly during NREM and waking immobility, and has similar waveform characteristics to hippocampal ripples. Furthermore, these oscillations appear to be coordinated with hippocampal ripples across behavioral states and during performance of a hippocampal-dependent memory task. This finding was facilitated by the scalability of our array, and establishes how this feature could be an important advantage in the design of neurophysiological experiments to understand functional topographic interactions among brain regions.

Disclosures: D. Khodagholy: None. **J.N. Gelinas:** None. **G. Buzsaki:** None.

Poster

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Program#/Poster#: 591.13/G25

Topic: B.10. Network Interactions

Support: EU-FP7-ERC-2013-Starting grant (No. 337075)

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Title: Sustained effects of a lifelong closed loop control of absence epilepsy in rats

Authors: *G. KOZAK[1], A. BERENYI[2,3];
[2]Dept. of Physiol., [1]Univ. of Szeged, Szeged, Hungary; [3]Neurosci. Inst., New York Univ., New York City, NY

Abstract: Epilepsy affects 1% of the population worldwide. Despite the development of drug therapies, still one-third of the patients remain unresponsive to any currently available anti-epileptic drug. The aberrant brain activity, which occurs in epilepsy, in principle, can be restored through electrical stimulation. In epilepsy, abnormal patterns emerge intermittently, and therefore, a closed-loop feedback brain control that leaves other aspects of brain functions unaffected is desirable. Formerly, it has been shown by our group that seizure-triggered, closed-loop transcranial electrical stimulation (TES) can dramatically reduce the duration of spike-and-wave (SW) episodes in a rodent model of generalized epilepsy. The long-term effects of such approach however have not been investigated yet due to the technical challenges of the reliable continuous seizure monitoring, automated detection and stimulation. In this study we developed

a custom designed non-supervised FPGA-based closed loop system, which automatically detected the onset and the internal pattern of each seizure from ECoG signal, and delivered temporally targeted transcranial electrical pulses to disintegrate the seizure related oscillations. To evaluate the long term effects of this on-demand transcranial seizure interruption, we divided chronically implanted freely moving Long Evans rats into three subgroups: on-demand stimulation, random stimulation and no stimulation group. All stimulated rats received the treatment up to 3 months continuously. We found that our closed loop approach is effective in terms of seizure duration reduction, which effect was immediate and did not deteriorate over long time. The animals did not show any overt behavioural change due to treatment. These findings promote the closed loop control as a safe and reliable alternative for seizure suppression in drug resistant patients.

Disclosures: G. Kozak: None. **A. Berenyi:** None.

Poster

591. Oscillations and Synchrony: Other III

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

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Topic: B.10. Network Interactions

Support: -FP7-ERC-2013-Starting grant (No.337075

Hungarian Academy of Sciences (LP2013-62/2013)

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NIH MH54671

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EMBO (ALTF 147-2015)

Title: Coordinated gamma inputs to CA1 place cells determine the strength of phase-precession

Authors: *A. FERNÁNDEZ RUIZ[1,2], A. OLIVA[1], G. A. NAGY[1], G. BUZSÁKI[2], A. BERÉNYI[1,2];
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Abstract: During the exploration of an environment most CA1 pyramidal cells fire in specific locations. These “place cells” have a plastic nature, varying their firing rate during successive

crossings through the same location. Another remarkable characteristic of hippocampal place cells is that in addition to this rate code they also express a temporal code. This temporal code exhibits a relationship between the position of the rat within the place-field and the timing of the place cell's spikes relative to the ongoing theta rhythm. This relationship arises from the gradual shift of cell firing to earlier phases of local theta oscillation as the animal travels through the place-field. It has been suggested that both intrahippocampal (CA3) and cortical (entorhinal -EC-) inputs contribute to place cell expression. However, the mechanisms by which those inputs interact or their relation with phase precession remains unknown.In previous work we developed a method to isolate CA3 and EC layer 3 (EC3) gamma inputs to the CA1 region in the behaving rat (Schomburg et al., 2014). We showed that EC3 fast gamma input (70 - 110 Hz) dominated at the theta peak and was followed by slower (30 - 60 Hz) CA3 input at the descending theta phase and a even faster (110 - 180 Hz) local gamma oscillation at the theta through. CA3 and EC3 inputs can compete or cooperate to modulate the firing of CA1 pyramidal cells and interneurons according to behavioral demands. In the present work we employed large-scale silicon probes (up to 512 channels) to record local field potentials (LFPs) and single-unit activity simultaneously in all hippocampal subfields during various navigational tasks and sleep. Most place cells displayed strong phase precession in the linear track. However a lap-by-lap analysis revealed that the magnitude of phase precession varied from trial-to-trial within a given session. The distribution of the relative strength of CA3 and EC3 gamma inputs to CA1 place cells followed a specific pattern along the place-field transversal. In the first half of the place-field, EC3 input dominated while CA3 input took over in the second half. This pattern was absent in laps without phase-precession. We propose that the coordination of CA3 and EC3 theta-modulated gamma inputs to CA1 place cells control the expression of phase-precession.
Schomburg EW, Fernández-Ruiz A, Berényi A, Mizuseki K, Anastassiou CA, Koch C, Buzsáki G. (2014) Theta phase segregation of input-specific gamma patterns in entorhinal-hippocampal networks. Neuron. 84:470-485
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Disclosures: **A. Fernández Ruiz:** None. **A. Oliva:** None. **G.A. Nagy:** None. **G. Buzsáki:** None. **A. Berényi:** None.

Poster

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Topic: B.10. Network Interactions

Support: MH107662

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NS90583

MH54671

MH107159

Title: Cortical read out of hippocampal neural sequences

Authors: *S. A. MCKENZIE, D. F. ENGLISH, G. BUZSÁKI;
NYUMC, New York, NY

Abstract: In the hippocampus, the order in which neurons fire maximally during movement is preserved on a faster time scales during active behavior and also during offline periods, such as sleep. However, the function of these sequences is unknown, in part due to a lack of information as to how downstream structures integrate such sequential activity. To address this issue, simultaneous recordings were performed in CA1 and its cortical output areas while spike timing of CA1 pyramidal cells was modulated on fine-time scales via focal ChR2 activation in behaving rats. First, in the absence of light stimulation, we observed tight coordination of sequences in CA1 and the subiculum during during linear track behavior. In both regions, sequences were organized by theta that began with representations of the track's beginning on the falling phase,represented current position at the trough and ended with the goal location at the rising phases. The degree of temporal compression varied as a function of distance away from these goals. Preliminary experiments show that low intensity, intra-hippocampal optical stimulation of pyramidal cell evoked local high frequency oscillations and cortical up states with spiking responses at a lag of about 70ms after CA1 stimulation. The efficacy of the cortical response correlated with the power and phase of cortical rhythms at the time of stimulation in the delta band and at 15-20 Hz. Surprisingly, stimulation of a second CA1 site before *or after* stimulation of the first blocked the cortical spiking response and upstate suggesting a competitive temporal or spatial summation downstream of the hippocampus. These results show that relative spike timing can strongly influence downstream spiking properties, a prerequisite for readout of endogenous sequences.

Disclosures: S.A. McKenzie: None. **D.F. English:** None. **G. Buzsáki:** None.

Poster

591. Oscillations and Synchrony: Other III

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NIH grant 1U01NS090583-01

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Title: Spatially focused, non-invasive, fast pulse electrical stimulation of the brain

Authors: *M. VOROSLAKOS[1,2], K. BRINYICZKI[3], T. ZOMBORI[3], B. IVANYI[3], G. BUZSÁKI[4], A. BERÉNYI[2,4];
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Abstract: Transcutaneous electric stimulation (TES) using weak currents has been used extensively in attempts to influence brain activity. In vitro and in vivo experiments in rodents and computational modeling suggest that the magnitude of voltage gradient of the induced electric field should exceed 1 mV/mm to directly influence brain networks. Evidence for direct neuronal effects of TES in the human brain is still lacking, mainly due to the saturation of the recording amplifiers by the large induced electromagnetic fields. For many therapeutic applications, it is desirable to affect neurons in a regionally constrained manner to reach maximum on-target effects and reduce side effects on unintended brain networks.
Here, we describe a spatially focused TES protocol, test it in rodents, determine the needed TES currents in human cadavers to achieve 1 mV/mm fields and demonstrate direct TES effects on alpha waves in human subjects. Using multiple site, rotating fast pulse (2.5 μs) stimulation, we demonstrate how transcranial electric fields can be focused to differentially activate single neurons in anesthetized rats. DC, AC and fast pulse stimulation was applied via Ag/AgCl electrodes directly to the skull or the scalp in human cadavers, while recording the three-dimensional distribution of electric fields in the brain, using >200 recording sites.
Scalp stimulation greatly reduced the generated intracerebral electric fields (>50% in cadavers)

and these measurements predicted that ~5 mA is needed to achieve 1mV/mm electric field gradient via scalp stimulation. Fast pulse stimulation allowed for simultaneous recording of scalp EEG in human subjects up to 9 mA. As predicted from the cadaver measurements, up to 4 mA stimulation failed to show detectable alterations but 7 mA and 9 mA integral-amplitude pulses significantly increased the amplitude of alpha waves. Currents above 4 mA stimulation induced multiple adverse effects, including dizziness, burning feeling and auditory effects.
Supported by the EU-FP7-ERC-2013-Starting grant (No. 337075), the 'Momentum' program of the Hungarian Academy of Sciences (LP2013-62/2013), the 'Excellence' program of the Hungarian Academy of Sciences (KEP-1.2/2014), NIH grants, 1U01NS090526-01, 1U01NS090583-01, R01MH102840

Disclosures: M. Voroslakos: None. **K. Brinyiczki:** None. **T. Zombori:** None. **B. Ivanyi:** None. **G. Buzsáki:** None. **A. Berényi:** None.

Poster

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Topic: B.10. Network Interactions

Support: MH107662

MH107396

NS90583

MH54671

Charles H. Revson Senior Fellowship in Biomedical Science

Title: Coordinated reactivations in the hippocampus-amygdala networks during slow-wave sleep

Authors: *G. GIRARDEAU, I. INEMA, G. BUZSAKI;
New York Univ. Langone Med. Ctr., New York, NY

Abstract: The hippocampus and the amygdala are brain areas thought to be critical for emotional memory consolidation. The hippocampus processes episodic and spatial memories while the amygdala signals emotional valence related to fear and reward. However, it is unknown how the two structures interact to associate an emotional event to a specific context. Since hippocampal memories are reactivated and consolidated during slow-wave sleep (SWS),

we hypothesized that sleep-related coordinated reactivations in the hippocampus-amygdala networks might sustain the formation of contextual emotional memories.
To test this, we designed a novel task in which rats learn the daily location of an aversive airpuff on a linear track where they're running for water rewards. We recorded large hippocampal and amygdala neuronal ensembles during training and extensive periods of sleep preceding and following training.
Our results show coordinated reactivations of hippocampus and basolateral amygdala (BLA) ensembles during slow-wave sleep (but not REM-sleep) following training. Interestingly, the BLA cells that contribute most to the reactivations are the ones that are positively modulated during hippocampal ripples. These findings suggest that the consolidation of contextual emotional memories might involve coordinated reactivations in hippocampal-amygdala networks during SWS hippocampal ripples.

Disclosures: G. Girardeau: None. **I. Inema:** None. **G. Buzsaki:** None.

Poster

591. Oscillations and Synchrony: Other III

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HFSP LT-000346/2009-L

Title: Sharp wave ripples stabilize hippocampal spatial map during learning

Authors: *L. ROUX[1], B. HU[2], R. EICHLER[3], E. STARK[4], G. BUZSÁKI[1];
[1]NYU Med. Center, Neurosci. Inst., New York, NY; [2]Dept. of Physiol., Third Military Med. University, Col. of Basic Med. Sci., Chongqing, China; [3]Departments of Neuroinformatics and Neurophysiol., Radboud Univ. Nijmegen, Donders Ctr. for Neurosci., Nijmegen, Netherlands;

[4]Dept. of Physiol. and Pharmacol., Tel Aviv University, Sackler Fac. of Medicine, Sagol Sch. of Neurosci., Tel Aviv, Israel

Abstract: Replay of behaviorally-relevant spike sequences in the hippocampus during sharp wave ripples (SPW-Rs) is thought to provide a crucial mechanism for memory consolidation. Indeed, the compressed temporal scale of place cell reactivations, combined with the strong synchronous activity associated with SPW-Rs, provides an ideal setting for synaptic modifications. Yet the role of SPW-Rs in stabilizing the hippocampal network during learning has never been tested directly. To address this question, we used the spatial information coded by place cells: we reasoned that if SPW-Rs are necessary for the network stabilization, disrupting neuronal activity specifically during those events would result in an altered mental representation of space ("cognitive map"). Mice performed a spatial memory task requiring daily learning of three goal locations (hidden rewards) on a familiar multi-well maze (Dupret et al., 2010). Similar to previous studies, SPW-Rs occurred regularly during reward consumption at the goal locations. We used online position tracking, high-density extracellular recordings, closed-loop feedback, and optogenetic stimulation to selectively suppress pyramidal cell activity in the CA1 region contingent upon detection of awake SPW-Rs, specifically at the goal locations. Silicon probe recordings, combined with focal light delivery, allowed us to simultaneously record "SPW-R-silenced" and “control” place cells (non-illuminated/non-silenced and/or place cells silenced with a delay relative to SPW-R occurrence). Following learning, control place cells typically maintained the location of their place fields and showed a significant increase in their spatial information content. In contrast, the place fields of SPW-R-silenced place cells were modified after learning (“destabilized”), and the quantity of spatial information carried by these neurons was unaltered. SPW-R silencing did not impact the firing rates or the proportions of place cells. These observations indicate that SPW-R-associated neuronal activity is necessary to "refine" and maintain hippocampal place fields upon learning. SPW-Rs could thus play a dual role, promoting both change and stability in the cognitive map.

Disclosures: **L. Roux:** None. **B. Hu:** None. **R. Eichler:** None. **E. Stark:** None. **G. Buzsáki:** None.

Poster

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NIH Grant MH54671

Title: Mapping rodent behavioral states onto the firing of dCA1 pyramidal cells

Authors: *J. D. LONG II[1], G. BUZSAKI[2];
[1]Neurosci. Inst., New York Univ., New York, NY; [2]Neurosci., New York Univ. Langone Med. Ctr., New York, NY

Abstract: The hippocampus is necessary for both episodic memory formation and spatial navigation. The central hypothesis of this work is that the unifying function of the hippocampus is to infer associations between sequences of behavior and their consequences. We predict neural correlates of specific behaviors exist within rodent dorsal CA1 hippocampus above and beyond spatial location and head direction. In this study, we utilized a cheeseboard maze, allowing the rats (n = 5) to form stereotyped, idiosyncratic behavioral plans while foraging for water reward. Next, the time-series of the subjects' behavioral output was quantified using a custom-made markerless motion capture system that generated accurate 3D kinematic tracking data of our subjects at 50 frames per second. Lastly, the behavioral states of the subjects were automatically identified using a unsupervised learning framework we have developed. This system enables us to calculate correlations between these behaviors and the time-series of neural data (all subjects were implanted with 6-8 shank silicon probes bilaterally in dCA1). We define behaviors as sequences of stereotyped dynamics in the kinematic data. We leverage recently developed techniques in machine learning to identify stereotyped dynamic features derived from the kinematic data (van der Maaten and Hinton 2008; Berman et al. 2014). To compose these features into models of behavior, we adapted the Bayesian Generative Model (BGM) framework of Lake et al. 2015 to our context. By first parsing the kinematic data into movement bouts, and then weakly labeling these with the learned features, BGMs were then used to leverage the statistics across all movement bouts to both improve the feature labeling and to produce parsimonious, quantitative compositions of the sequence of features making up a putative behavior. With this framework in place, we applied analysis methods in systems neuroscience to search for neural correlates of rodent behavior in dCA1 hippocampus. Reverse correlation is used to determine whether the firing of individual neurons map onto specific behaviors. Ensemble decoding techniques are used to determine whether information about the moment to moment variations in the subjects' behaviors reflected by the population activity. This ongoing research may offer new insights into the function of the hippocampus.

Disclosures: J.D. Long II: None. **G. Buzsaki:** None.

Poster

591. Oscillations and Synchrony: Other III

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Topic: B.10. Network Interactions

Support: Ministère de la Recherche

ENP

Labex

Title: Role of network functional connectivity in the formation of theta sequences

Authors: *C. DRIEU, R. TODOROVA, M. ZUGARO;
CIRB, Collège De France, Paris, France

Abstract: The hippocampus codes for spatial and episodic memories. Hippocampal 'place' cells are selectively activated in restricted locations of the environment ('firing fields'). Because firing fields overlap in space, as the animal traverses successive fields the spikes emitted by place cells appear intermingled in time. However, during single cycles (~100ms) of the theta oscillation (7-10Hz), these cells fire in a stereotyped order, paralleling the order of the fields within the trajectory. Such 'theta sequences' are thought to be related to episodic-like memory.
Two frameworks have been proposed to account for the formation of theta sequences. The first theory posits that sequences result from delays in propagation of spiking activity between asymmetrically connected cell assemblies. In the second model, external inputs trigger activity in independent cell assemblies. Due to phase precession, each assembly advances its firing in successive theta cycles, which results in theta sequences. Here, asymmetric connections between assemblies results from, rather than cause, theta sequences.
To test and contrast these hypotheses, we have passively transported rats on a model train ('passive' condition). As previously documented, this perturbed phase precession. In the control experiments, rats were trained to run on a miniature treadmill while transported on the train ('active' condition), a condition in which we have previously reported that cells phase precess and form theta sequences. We thus asked whether theta sequences would be disrupted in the passive condition. Place cells and LFP were recorded during three successive sessions: PASSIVE-1, ACTIVE, PASSIVE-2. We compared place cell spatial properties and dynamics between the three conditions.
Spatial specificity and theta modulation of place cell activity decreased during both passive sessions compared to the active session. Fields often shifted between PASSIVE-1 and ACTIVE, but subsequently remained stable between ACTIVE and PASSIVE-2. The proportion of significant theta sequences increased two-fold during ACTIVE compared to PASSIVE-1. This

appears inconsistent with the notion that pre-established connectivity between cell assemblies underlies theta sequences. Intriguingly, we found a similar difference between ACTIVE and PASSIVE-2. The sequences formed during ACTIVE were not stabilized and preserved during the subsequent passive session, an observation at odds with the idea that theta sequences would induce changes in synaptic connectivity between cell assemblies. Thus, our results so far appear at least partly inconsistent with both theoretical models of theta sequences.

Disclosures: C. Drieu: None. **R. Todorova:** None. **M. Zugaro:** None.

Poster

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Topic: B.10. Network Interactions

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MH54671

Title: Optogenetically assisted identification of spike transmission between pyramidal neurons and interneurons *In vivo*

Authors: *D. F. ENGLISH[1], S. MCKENZIE[1], K. KIM[2], E. YOON[2], G. BUZSAKI[1]; [1]Neurosci., NYU Neurosci. Inst., New York, NY; [2]Electrical Engin. and Computer Sci., Univ. of Michigan, Ann Arbor, MI

Abstract: Hippocampal microcircuits have been the subject of intense investigation for decades. A major goal is to understand the cellular-synaptic mechanisms of precise spike timing of hippocampal neurons, which is required for temporal coding. One such mechanism is the spike transmission between pairs of neurons. Studies of synaptic and spike transmission between identified presynaptic and postsynaptic neurons are typically performed in reduced preparations such as acute brain slices. In vivo studies typically sacrifice control of neuronal activity with single cell resolution in order to study an intact system. In such *in vivo* experiments synaptic coupling can be observed as short (1-2ms) latency peaks in spike train cross-correlations between PYR and INT. However, these findings are limited by the fact that common inputs to both the PYR and INT could underlie part of the fast time-scale peak. This technical shortcoming

can now be overcome due to recent advances in high-channel count silicon probes equipped with blue light emitting micro-LEDs (Si-uLED probe; Wu et al. 2015) which can be combined with optogenetics to maintain the benefits of an *in vivo* preparation while gaining single cell control and identification. We took advantage of this technology to investigate monosynaptic interactions between pyramidal neurons (PYR) and GABAergic interneurons (INT) in the CA1 region of the hippocampus in freely sleeping and behaving mice. To disengage the PYR spiking from network activity and thus common input we optically elicited spiking in PYR using CaMKII:ChR2 mice and quantified the strength of spike transmission in pairs of PYR and INT. Preliminary data demonstrate that both spontaneous and experimentally induced PYR spikes drive spiking in postsynaptic INT at monosynaptic latencies. Future experiments will determine the dynamic range and temporal dynamics of these connections and their regulation by brain state and behavioral conditions. Wu F, Stark E, Ku PC, Wise KD, Buzsáki G, Yoon E. Monolithically Integrated μLEDs on Silicon Neural Probes for High-Resolution Optogenetic Studies in Behaving Animals. Neuron. 2015 Dec 16;88(6):1136-48

Disclosures: **D.F. English:** None. **S. McKenzie:** None. **K. Kim:** None. **E. Yoon:** None. **G. Buzsaki:** None.

Poster

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Title: CA2 activity precedes ripples in CA1

Authors: ***A. OLIVA GONZÁLEZ**[1], A. FERNANDEZ-RUIZ[1,2], G. BUZSÁKI[2], A. BERÉNYI[1,2];

[1]Physiol. Dept., Univ. of Szeged, Szeged, Hungary; [2]Neurosci. Inst. and Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Sharp-wave ripples (SPW-Rs) are network oscillations in the mammalian hippocampus that are implied in memory consolidation as shown by observational and interventional experiments. However, the mechanism of their generation remains unclear. SPW-R complex consists of two components: the sharp wave (SPW) and the ripple. The sharp wave is a negative deflection in the extracellular potential that reflects the depolarization of the apical dendrites of CA1 pyramidal cells as a result of the synchronous discharge of the CA3 axonal input to those dendrites, and a ~140 Hz ripple at the pyramidal cell body layer representing a network response of CA1 pyramidal cells and interneurons to the strong synchronous drive. While the CA3 region has been implicated in the generation of the SPWs, the role the adjacent CA2 region has been neglected so far. However, the CA2 region mirrors some aspects of CA3: the recurrent collaterals of CA2 pyramidal neurons are as dense as those of CA3a neurons, and CA2 also innervates CA1, leaving open the possibility that CA2 could be involved in ripple generation as well. In this study we examined whether the activity of CA2 region plays a role in SPW-R burst initiation, which has not been investigated until now. We employed high-density silicon-probes (256 channels) to record both LFP and unit firing from all layers of the CA1-CA2-CA3 regions in the dorsal hippocampus in rats during behavior and sleep. The location of the CA2 region was assessed by immuno labeling using the CA2-specific marker PCP4. We found that synchronous activation of neuronal ensembles in the CA2 region preceded SPW-R-related population activity in CA3 and CA1 regions. Deep CA2 neurons showed a gradual increase in activity prior to ripples and become strongly suppressed whenthe population activity built up in CA3-CA1 neurons (*ripple ramp cells*). Superficial CA2 cells organized into a population burst prior to the activity surge in CA3-CA1 (*phasic cells*). The population activation spreads through CA3b and c and finally to CA1 stratum radiatum where it elicits an excitatory SPW. Although CA2 neurons contribute to SPW-Rs during both sleeping and waking, their contribution to wake events is stronger. These results implicate the CA2 region as an initiation zone of SPW-Rs. This work was supported by EU-FP7-ERC-2013-Starting grant (No.337075), the ‘Momentum’ Program of the Hungarian Academy of Sciences (LP2013-62/2013), NIHNS034994, NIH MH54671, NIH NS074015, Fundación La Caixa, EMBO (ALTF 147-2015).

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Poster

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March of Dimes

Title: Interictal epileptiform discharges induce hippocampal-cortical coupling in temporal lobe epilepsy

Authors: *J. GELINAS, D. KHODAGHOLY, O. DEVINSKY, G. BUZSAKI;
New York Univ., New York, NY

Abstract: Interactions between the hippocampus and the cortex are critical for memory. Interictal epileptiform discharges (IEDs) identify epileptic brain regions and can impair memory, but the mechanisms by which they interact with physiological patterns of network activity are mostly undefined. We show in a rat model of temporal lobe epilepsy that spontaneous hippocampal IEDs correlate with impaired memory consolidation, and that they are precisely coordinated with spindle oscillations in the prefrontal cortex during nonrapid-eye-movement (NREM) sleep. This coordination surpasses the normal physiological ripple-spindle coupling and is accompanied by decreased ripple occurrence. IEDs also induce spindles during rapid-eye movement (REM) sleep and wakefulness—behavioral states that do not naturally express these oscillations—by generating a cortical 'down' state. In a pilot clinical examination of four subjects with focal epilepsy, we confirm a similar correlation of temporofrontal IEDs with spindles over anatomically restricted cortical regions. These findings imply that IEDs may impair memory via the misappropriation of physiological mechanisms for hippocampal-cortical coupling, which suggests a target for the treatment of memory impairment in epilepsy.

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Poster

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Title: Transformation of head-direction signal into spatial code

Authors: *A. PEYRACHE[1,2], G. BUZSAKI[2];
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Abstract: The head-direction (HD) system forms the building block of the navigation system. Spatial coding, represented for example by place and grid cells, must result from the integration of multiple sources of information, distributed over large class of sensory features, in particular vestibular signals. The mechanisms enabling such computation remain largely unknown. It is often assumed that the HD signal is primarily used, along with speed information, to update the estimate of the current location by path integration. Here we show that the HD signal may be transformed directly into a spatial code under behavioral constraints. In the antero-dorsal nucleus of the thalamus, relaying the signal to the cortex, HD neurons convey high level of spatial information that results from behavioral bias during exploration. In the post-subiculum, the main cortical stage of HD signal processing, the amount of spatial information conveyed by HD neurons is increased by the combination of the HD signal with other sensory modalities. In addition, the hippocampus may receive direct sensory inputs from the HD system, thus participating directly in the generation of a spatial code. These findings demonstrate a simple integration principle in the navigation system that transforms vestibular inputs into spatial information.

Disclosures: **A. Peyrache:** None. **G. Buzsaki:** None.

Poster

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MH54671

Title: Exploration of Hippocampal-Lateral Septal interactions across behavioral states

Authors: *D. TINGLEY[1,2], G. BUZSÁKI[2];
[1]New York Univ., New York, NY; [2]NYU Neurosci. Inst., New York, NY

Abstract: Relative to other major output structures of the hippocampal formation (HPC) the lateral septal complex (LSC)- its function, behavioral correlates, and physiological relationship with HPC - remains to a great extent unexplored. Lesion, anatomy, and a handful of *in vivo* physiology experiments have demonstrated that the LSC plays a role in contextual, social, and affective aspects of cognitive function. Here we investigate the role of the lateral septum in navigation and contextual encoding with simultaneous HPC/LSC in vivo recordings. LSC neurons were phase-locked to the theta rhythm and modulated by hippocampal sharp wave ripples. High frequency oscillations (80-160 Hz) were also present in the LSC local field potential, were accompanied by an increase in LSC firing rates, and traveled in a directional manner within LSC. Surprisingly, a large number of HPC/LSC neuron pair cross-correlograms showed suppressed spiking with a ~20-40 millisecond delay, suggesting that HPC output has an indirect inhibitory effect on LSC activity, possibly through feedforward inhibition. During goal directed spatial navigation a subset of LSC neurons showed a novel form of phase precession relative to HPC theta oscillations. They precessed over long segments of maze runs (up to 3 meters), were independent of the heading and velocity of the animal, and were not accompanied by large firing rate changes. An additional subset of LSC neurons were found to be strongly modulated by a specific local context, independent of allocentric location and distal cues. These neurons fired almost exclusively when the animal occupied its home cage, and were greatly suppressed during exposure to novel environments (with or without task demands), familiar environments (up to one month of daily exposure), and home cages with clean bedding or the bedding from other rats. These data suggest that certain contexts may hold privileged neural representations in the LSC. Experiments in progress will continue to investigate the mechanisms underlying these phenomena and whether they reflect local computations, or interactions with other structures such as the hippocampal formation.

Disclosures: D. Tingley: None. **G. Buzsáki:** None.

Poster

591. Oscillations and Synchrony: Other III

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 591.26/G38

Topic: B.10. Network Interactions

Support: NIH K08 DA036657

NARSAD Young Investigator Award

Title: Selective hippocampal-accumbal plasticity is a key substrate of cocaine conditioned place preference

Authors: *L. L. SJULSON, A. CUMPELIK, D. CASSATARO, G. BUZSÁKI;
NYU Sch. of Med., New York, NY

Abstract: Cocaine conditioned place preference (CPP) is one of the simplest models of a cocaine-addiction related behavior, but its underlying neural mechanisms are not understood. To test the hypothesized role of plasticity of the projection from hippocampus to nucleus accumbens, we performed simultaneous dual site silicon probe recording in mice undergoing a cocaine CPP protocol. Our results suggest that CPP is associated with selective strengthening of hippocampal-accumbal synapses arising from place cells encoding the cocaine-paired location. Further preliminary results suggest differential effects of synapses onto D1- vs D2-positive cells in accumbens, as well as behavioral evidence indicating a causal role for this plasticity in CPP.

Disclosures: L.L. Sjulson: None. **A. Cumpelik:** None. **D. Cassataro:** None. **G. Buzsáki:** None.

Poster

591. Oscillations and Synchrony: Other III

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 591.27/G39

Topic: B.10. Network Interactions

Support: NARSAD Young Investigator Award 2014

Title: Unit and field potential changes in rats after antidepressant dose ketamine

Authors: *B. O. WATSON[1], J. P. GREENE[2], M. DING[3], G. BUZSAKI[4];
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Abstract: Low dose ketamine has gathered interest as a rapid-acting antidepressant with efficacy on many patient groups including those with unipolar, bipolar and treatment resistant-depression. Here we explore the neurophysiologic correlates of anti-depressant dose ketamine in rats using silicon probe recordings in the dorsal hippocampus and frontal cortical regions. Clinical findings indicate that ketamine may work with a truly unique mechanism of action relative to other antidepressant treatments. Firstly it is known to have unique pharmacologic properties since it acts as an NMDA antagonist, an opioid receptor agonist and has cholinergic and dopaminergic effects among others. Perhaps more importantly, it is able to alleviate depressive symptoms within hours and that effect lasts for days. Understanding the mechanism of action of this uniquely effective medication has the potential to teach us a great deal about depression treatment approaches fundamentally. We have given 10mg/kg ketamine intraperitoneally to 7 adult male Long Evans rats while recording from frontal cortex and dorsal hippocampus. We find that during the period immediately following ketamine animals are hyperlocomotive for 25-45 minutes each before reliably going to sleep. During the period of hyperactivity we see increased average spike rates among both putative excitatory and putative inhibitory neurons. This is in contrast to published findings from similar experiments using other NMDA antagonists (1). In many cases we see specific subsets of neurons that are particularly responsive to ketamine. We also see increased gamma oscillatory power. We observe a temporary increase in the coefficient of variation of population firing rates, decreased bursting activity and an increase in excitatory-to-inhibitory ratio. However, none of these changes deviate from what would be predicted simply by the increase in locomotion by the animal, given the relationship between each of these metrics and times of movement. It is possible that persistent elevated activity in the frontal cortex lasting ten of minutes is a mechanism itself for network change due to ketamine, consistent with optogenetic stimulation results (2).
1. Homayoun H, Moghaddam B. J Neurosci. 2007 Oct 24;27(43):11496-500. 2. Fuchikami M, Thomas A, Liu R, Wohleb ES, Land BB, DiLeone RJ, Aghajanian GK, DumanRS. Proc Natl Acad Sci U S A. 2015 Jun 30;112(26):8106-11

Disclosures: **B.O. Watson:** None. **J.P. Greene:** None. **M. Ding:** None. **G. Buzsaki:** None.

Poster

591. Oscillations and Synchrony: Other III

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 591.28/G40

Topic: B.10. Network Interactions

Support: ISRAEL SCIENCE FOUNDATION Grant No. 754/14

ISRAEL SCIENCE FOUNDATION Grant No. 51/11

Israeli Ministry of Science, Technology and Space

Title: Near-critical dynamics in stimulus-evoked activity of the human brain and its relation to spontaneous resting-state activity

Authors: *O. SHRIKI[1], A. GOLDSTEIN[2], O. ARVIV[2];
[1]Ben-Gurion Univ., Beer-Sheva, Israel; [2]Bar-Ilan Univ., Ramat-Gan, Israel

Abstract: In recent years, numerous studies have found that the brain at resting-state displays many features characteristic of a critical state. Here we examine whether stimulus-evoked activity can also be regarded as critical. Additionally, we investigate the relation between resting-state activity and stimulus-evoked activity from the perspective of criticality. We found that cortical activity measured by MEG is near critical and organizes as neuronal avalanches at both resting-state and stimulus-evoked activities. Moreover, a significantly high intra-subject similarity between avalanche size and duration distributions at both cognitive states was found, suggesting that the distributions capture specific features of the individual brain dynamics. When comparing different subjects, a higher inter-subject consistency was found for stimulus-evoked activity than for resting-state. This was expressed by the distance between avalanche size and duration distributions of different participants, and was supported by the spatial spreading of the avalanches involved. During the course of stimulus-evoked activity, time-locked to the stimulus onset, we demonstrate fluctuations in the gain of the neuronal system, and thus short time-scale deviations from the critical state. Nonetheless, the overall near-critical state in stimulus-evoked activity is retained over longer time-scale, in close-proximity and with a high correlation to spontaneous (not time-locked) resting-state activity. Spatially, the observed fluctuations in gain manifest through anti-correlative activations of brain sites involved, suggesting a switch between task-negative (default mode) and task-positive networks and assigning the changes in excitation-inhibition balance to nodes within these networks. Overall, this study offers a novel outlook on evoked activity through the framework of criticality.
Reference: Arviv O., Goldstein A., and Shriki O., Near-critical dynamics in stimulus-evoked activity of the human brain and its relation to spontaneous resting-state activity. Journal of Neuroscience, 35: 13927-13942, 2015.

Disclosures: **O. Shriki:** None. **A. Goldstein:** None. **O. Arviv:** None.

Poster

591. Oscillations and Synchrony: Other III

Location: Halls B-H

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Program#/Poster#: 591.29/G41

Topic: B.10. Network Interactions

Support: NIMH Intramural Research Program

Maryland Biophysics Graduate Program

Title: Oscillations and neuronal avalanche shape in spontaneous cortical activity

Authors: ***S. R. MILLER**[1,2,3], S. YU[3], D. PLENZ[3];
[1]UMCP, Washington, DC; [2]Inst. for Physical Sci. and Technology, Biophysics, Univ. of Maryland Col. Park, College Park, MD; [3]Lab. for Systems Neurosci., Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Spatiotemporal synchronization in the form of phase-locked gamma oscillations (i.e. at a time scale of ~30 ms) has been robustly found during cortical processing. Such synchronization based on oscillations seems in apparent contradiction to an alternative form of spatiotemporal synchronization consistently identified as neuronal avalanches. Avalanches are intermittent spatiotemporal bursts of activity governed by power laws and thus lack spatial or temporal scale. Previous work demonstrated that the power law in avalanche sizes embeds the waxing and waning of nested cortical oscillations (Gireesh and Plenz, 2008). However, the temporal domain of avalanches and its relation to oscillations has not been addressed. Here, we utilize a shape collapse function, which tests for scale-invariance in avalanche shapes of different durations, and relate the quality of this collapse to gamma oscillations.
Ongoing local field potential (LFP) fluctuations were recorded in three awake Macaque monkeys, each with two chronically implanted high-density arrays in superficial cortex (10x10 electrodes; 400 μm interelectrode distance; 2 premotor, 4 prefrontal). During recordings the monkeys were sitting alert without performing any particular task (26 hours total; ~4 hrs/array; 4 - 23 recording sessions distributed over 5-74 days). LFPs (1 - 100 Hz) were thresholded (-2 SD) for each electrode and assembled into peak LFP time rasters (>70 channels/raster). Avalanches were extracted at successively increasing time bins Δt (0.5 ms - 30 ms). For each temporal resolution, avalanche size and duration distributions were calculated, shapes were averaged by duration, and the collapse function was optimized with respect to the scaling exponent.
Our two main findings suggest gamma oscillations provide a specific temporal scale to neuronal

avalanches. First, critical exponents calculated from avalanche probability distributions underestimated theoretical values, yet were a better predictor than theory for the collapse-optimized scaling exponent. Second, prominent oscillatory activity was associated with heterogeneous avalanche shapes and a failure of shape collapse. This result was found only for the range of temporal resolutions (2 ms < Δt < 15 ms) that resolved multiple gamma-cycles without overly coarse-graining fine LFP waveform features. We thus conclude that the cortex is able to balance oscillations with avalanche activity. In particular, gamma oscillations leave an 'imprint' on the lifetime distribution of avalanches when studied at gamma-resolvable scale, but not otherwise.

Disclosures: **S.R. Miller:** None. **S. Yu:** None. **D. Plenz:** None.

Poster

591. Oscillations and Synchrony: Other III

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 591.30/G42

Topic: B.10. Network Interactions

Title: Avalanche dynamics during processing of visual stimuli in the awake mouse

Authors: *T. L. RIBEIRO[1], S. SESHADRI[1], J. MISHLER[1], D. WINKOWSKI[2], P. KANOLD[2], D. PLENZ[1];
[1]Section on Critical Brain Dynamics LSN/NIMH, NIH, Bethesda, MD; [2]Dept. of Biol., Univ. of Maryland, College Park, MD

Abstract: Neuronal avalanches are spatiotemporal clusters of activity that propagate in superficial cortical layers. They have been considered evidence of critical dynamics as their sizes, defined as the number of neurons composing the cluster, are power-law distributed and therefore scale-free. In theory, criticality optimizes stimulus sensitivity, information transmission, computational capability, and mnemonic repertoires size. Yet, the stability of this dynamical regime during external stimulus remains unanswered. Here we study the role of functionally different sub populations, in particular cells with different orientation preference in the primary visual cortex (V1), in avalanche dynamics.We employed two-photon imaging to measure spiking activity in pyramidal cells from V1 of head-fixed mice positioned on a running wheel. Viral-injection into cortical layer 2/3 of wild-type mice resulted in robust expression of the genetically encoded calcium indicators (GECIs) YC2.6 or GCaMP6s after about 2 weeks. A chronic cranial window was centered over V1 and mice were subjected to passive viewing of drifting bars at 16 angles (4 s each, randomly chosen) at maximum contrast interspaced by 4 s of a gray screen. During stimulation, mice could self-initiate locomotion. Pupil diameter was

assessed continuously to measure the animal's state of arousal. About 70 neurons were recorded consistently in a ~560 x 560 μm area at a frame rate of ~30 Hz. Spiking increased moderately during locomotion and sub populations of neurons were identified to have preferred orientations. We analyzed neuronal avalanche statistics in those populations with and without visual stimulus present, separating periods according to state of arousal. Power law statistics was found to be robustly maintained during stimulation independent of orientation. When directionally selective neurons were analyzed separately we also observed power law statistics, similarly to what was seen for non-selective cells. Strikingly, these results remain even when only periods of stimulation are considered for the analysis, regardless of the specific orientation presented. In conclusion, neurons responsible for processing different characteristics of a visual stimulus follow the same dynamical rules as those which do not respond to that same feature.

Disclosures: T.L. Ribeiro: None. **S. Seshadri:** None. **J. Mishler:** None. **D. Winkowski:** None. **P. Kanold:** None. **D. Plenz:** None.

Poster

592. Epilepsy: Interneurons, Cell Therapies, and Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 592.01/G43

Topic: B.11. Epilepsy

Title: Leveraging iPSC-derived cortical neurons harboring known epilepsy mutations to advance personalized medicine

Authors: *C. B. CARLSON, M. MCLACHLAN, B. MELINE, C. MCMAHON, T. BURKE, S. DELAURA, E. JONES, K. MANGAN;
Cell. Dynamics Intl., Madison, WI

Abstract: Epilepsy is a neurological condition caused by disturbances in the electrical activity of the brain manifested through multiple etiologies. Over 65 million individuals suffer from epilepsy and one-third of these individuals live with uncontrollable seizures because there are no known pharmacological treatments to date. A portion of this population is accounted for by single-gene epilepsy disorders resulting from mutations within sodium, potassium, or inhibitory ion channels. With recent advances in personalized medicine, there is hope not only for diagnosis but also for treatment options for these individuals. Central to this vision is induced pluripotent stem (iPS) cell technology, which provides a platform to increase our understanding of how single-gene mutations result in disease states. Here we illustrate how human iPS cell-derived cortical neurons can be used to highlight the "disease-in-a-dish" approach to drug development and can act as a springboard to discovering new therapies. We have genetically engineered iPS

cells with single-gene mutations that result in Dravet Syndrome (SCN1A knockout), autosomal-dominant nocturnal frontal lobe epilepsy (KCNT1 P924L) or childhood absence epilepsy (GABRG2 R43Q). Human cortical neurons were derived from these iPS cell lines and the resulting phenotypes were examined by various methods. Here we present morphological data (neurite outgrowth and branching) and functional data (electrophysiological MEA or calcium flux) comparing "healthy" (wild-type) vs. edited (SCN1A, KCNT1, or GABRG2 mutations) neurons illustrating hyperactive phenotypes correlated to the epileptic genotypes. Furthermore, we show examples of selective pharmacology that attenuates these observed "epileptic" phenotypes. The ability to engineer isogenic wild-type and disease-associated alleles by genome editing of human iPSC-derived neurons provides unprecedented access to in vitro models of neurological disorders. Collectively our results showcase how iPSC technology can be leveraged in the personal medicine space.

Disclosures: C.B. Carlson: A. Employment/Salary (full or part-time): Cellular Dynamics International. **M. McLachlan:** A. Employment/Salary (full or part-time): Cellular Dynamics International. **B. Meline:** A. Employment/Salary (full or part-time): Cellular Dynamics International. **C. McMahon:** A. Employment/Salary (full or part-time): Cellular Dynamics International. **T. Burke:** A. Employment/Salary (full or part-time): Cellular Dynamics International. **S. DeLaura:** A. Employment/Salary (full or part-time): Cellular Dynamics International. **E. Jones:** A. Employment/Salary (full or part-time): Cellular Dynamics International. **K. Mangan:** A. Employment/Salary (full or part-time): Cellular Dynamics International.

Poster

592. Epilepsy: Interneurons, Cell Therapies, and Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 592.02/G44

Topic: B.11. Epilepsy

Title: Altered expression of KCC2 in GABAergic interneuron contributes prenatal stress-induced epileptic spasms in infant rat

Authors: H. KWON[1], *J. KANG[2], H. BAEK[3], J. PARK[3], D. KIM[4];
[1]Med. science, [2]Pediatrics, [3]Physiol., [4]Anat., Chungnam Natl. Univ., Daejeon, Korea, Republic of

Abstract: Long-term stress during pregnancy causes neurologic deficits to offspring with altered gamma-aminobutyric acid (GABA) system in the brain. However, it is not clear how prenatal stress affects the maturing GABAergic interneurons and the resulting abnormalities in infantile

seizures. Here, we showed that prenatal stress alters the maturation of GABA inhibitory system using a seizure model induced by prenatal stress. Prenatal stress with betamethasone or acute immobilization stress (AIS) on gestational day 15 increased the seizure susceptibility to N-methyl-D-aspartate-triggered spasms on postnatal day 15. The expression of GABA was lower in the prenatally stressed group, which compromise the decrease of glutamate decarboxylase 67-immunopositive cells. Prenatal stress markedly decreased the expression of K^+/Cl^- co-transporter (KCC2) in the cortex. GABA induced membrane depolarization demonstrated prenatal stress models had significant higher membrane depolarization compared to control. GABA increased KCC2 expression in cultured cortex-containing slices. Taken together, our results showed that prenatal stress with betamethasone or AIS altered the maturation of GABAergic progenitors and resulted in the lack of GABA input, which in turn, decreased KCC2 expression and lowered seizure threshold. We conclude that delayed GABA excitatory/inhibitory shift would render the cortical neuronal circuit more susceptible to excitatory input in prenatal stress induced seizure.

Disclosures: **H. Kwon:** None. **J. Kang:** None. **H. Baek:** None. **J. Park:** None. **D. Kim:** None.

Poster

592. Epilepsy: Interneurons, Cell Therapies, and Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 592.03/G45

Topic: B.11. Epilepsy

Support: NIH RO1 NS075366

Title: The role of tonic inhibition in shaping the firing characteristics of cortical pyramidal neurons in the y2R43Q mouse model of absence epilepsy

Authors: *J. A. PFAMMATTER, C. P. MAHANT, M. V. JONES;
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Abstract: Absence epilepsy involves generalized spike-wave discharges (SWDs) concurrent with behavioral arrests. One cause of absence epilepsy is a mutation in the γ2 subunit (γ2R43Q) of the $GABA_A$ receptor. Although the mechanism remains unknown, 'tonic' inhibition (TI), a constant hyperpolarization of neurons by extrasynaptic GABA that regulates neuronal firing, may play an important role. We previously showed that this mutation results in a reduction of $GABA_A$ receptor subunits that mediate TI in the cortex and thalamus of C57Bl6 γ2R43Q knock-in mice (RQ), and these mice lack tonic inhibition in these regions. We also previously showed that low dose ganaxolone, a selective potentiator of δ subunit-containing $GABA_A$ receptors, can exactly restore the TI missing in RQ cortical cells and reduce SWD frequency, and L655,708, an

inverse agonist of α5 subunit-containing $GABA_A$ receptors, can decrease TI in cortical cells of wild type mice (RR) and causes SWDs. We thus hypothesize that layer II/III pyramidal neurons of RQ somatosensory cortex, which lack TI, will have altered firing properties when stimulated with current steps in patch-clamp experiments. We further predict that restoration of TI in pyramidal neurons of RQ mice, with ganaxolone will restore normal firing properties, whereas blocking TI in RR neurons with L655,708 will partially mimic the firing properties in RQ mice. Preliminary results confirm that pyramidal cells in the cortex of RR mice (n=7) have a 6.4±0.4pA (mean±se) tonic current that is reduced to 1.45±0.52pA in RQ cells (n=6; $p \leq 0.012$). Surprisingly, RQ pyramidal cells regularly fired fewer action potentials (maximum 10.2±1.7; n=20) than RR cells (14.8±2.9; n=17; $p<0.19$) in response to current steps ranging from -100 to 300pA, although this result was not significant. Additionally, the duration of action potentials (width at half maximum) trended towards being longer in RR cells (3.98ms) than in RQ cells (2.92ms) at the current which elicited half-maximal spiking ($p<0.092$). Preliminary results are confounded by high cell-to-cell variability, possibly due to heterogeneity of recorded cell types. Ongoing efforts to more effectively compare RR and RQ cells involve paired measurements of cell responses before and after the addition of drug and immunofluorescent categorization of cell types. Ultimately, a better understanding of the role of the loss of TI in pyramidal cell function will help us understand the mechanisms underlying absence epilepsy in patients with compromised inhibition.

Disclosures: J.A. Pfammatter: None. **C.P. Mahant:** None. **M.V. Jones:** None.

Poster

592. Epilepsy: Interneurons, Cell Therapies, and Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 592.04/G46

Topic: B.11. Epilepsy

Support: NIH NS079214

NIH NS096976

Title: Epilepsy and behavioral comorbidities in a zebrafish model for Dravet syndrome

Authors: *B. GRONE, S. C. BARABAN;
Dept. of Neurolog. Surgery, Univ. of California, San Francisco, San Francisco, CA

Abstract: Dravet syndrome (DS) is a catastrophic epilepsy of childhood caused by mutations in SCN1A, a voltage-gated sodium channel. In addition to early-life generalized seizures, comorbid

conditions such as movement disorder, sleep disturbance, anxiety, early fatality and cognitive decline are common. To study the functional consequences of SCN1A mutations, we use zebrafish with a loss-of-function mutation in *scn1lab*, one of two zebrafish *scn1a* gene paralogs. Homozygous *scn1lab*$^{s552/s552}$ mutant larvae exhibit early-life seizures, metabolic deficits, and early death. In order to establish zebrafish as a model for quantifying comorbid conditions in DS, we carried out a battery of *in vivo* assays with *scn1lab*^{s552} mutants between 3 and 6 days post-fertilization (dpf). To study motor activity during a seizure, we used high-speed video imaging with simultaneous EEG recording in head-fixed zebrafish. In mutants (n=8), long-duration ictal events were associated with high-velocity, complex sinusoidal tail deflections >50 degrees and lasting 600-1200 msec; interictal events were associated with briefer tail movement and smaller angle deflections. To study anxiety, we used locomotion tracking to monitor exploratory behavior an open field arena. Mutants exhibited significantly impaired exploratory behavior, with increased time spent freezing and time spent in the periphery. To evaluate nighttime arousal disturbances, we tracked larvae in 96-well plate format for 24 hours. Locomotor activity during night (sleep phase) was higher in mutants compared to controls. To assess cardiac function, we measured heart rate using video recordings. No significant differences in heart rate compared to controls were observed. We also quantified the distribution of onset of fatality, which occurs in the first 14 days postfertilization. Our results demonstrate conserved features of movement disorders, anxiety, sleep disturbances, and early fatality in *scn1lab* mutant zebrafish.

Disclosures: B. Grone: None. **S.C. Baraban:** None.

Poster

592. Epilepsy: Interneurons, Cell Therapies, and Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 592.05/DP03 (Dynamic Poster)

Topic: B.11. Epilepsy

Support: CIHR

NSERC

Title: Optogenetic kindling of neocortex elicits seizures

Authors: *E. CELA[1,2,3], A. CHUNG[1], T. WANG[1], P. J. SJÖSTRÖM[1,2];
[1]Neurol. and Neurosurg., Montreal Gen. Hosp., Montreal, QC, Canada; [2]Ctr. for Res. in Neurosci., Montreal, QC, Canada; [3]Integrated Program in Neurosci., Montreal, QC, Canada

Abstract: How seizures arise in the otherwise healthy brain remains poorly understood. One way of addressing this is to unravel the circuit changes that are associated with seizure initiation. To this end, we sought to test the hypothesis that seizures can eventually be initiated in healthy mice solely by repeatedly driving pathological activity in a genetically defined subset of neocortical pyramidal cells. We developed a novel kindling paradigm based on repeated optogenetic stimulation of primary motor cortex (M1). Channelrhodopsin-2 (ChR2) was expressed in M1 of male C57BL/6J mice by stereotactically injecting AAV-CaMKIIa-hChR2-E123T/T159C-p2A-EYFP bihemispherically. After 21 days of recovery and ChR2 expression, animals were kindled by repeatedly illuminating M1 in 3-second-long 50-Hz burst every 48 hours using a 445-nm laser. Animals were monitored by EEG and video during each session. We observed seizures eventually occurring in 6 out of 6 animals after 13 sessions. Seizures were defined as EEG power exceeding background levels by two standard deviations for longer than 3 seconds. We quantified their duration using EEG recordings, and severity using a modified Racine scale. We found that seizure duration ($r=0.52$, $p<0.001$, $n=4$), severity ($r=0.59$, $p<0.001$, $n=4$), as well as the number of seizures ($r=0.48$, $p<0.001$, $n=6$) increased with session, while seizure threshold was decreased ($r=-0.59$, $p<0.001$, $n=4$). We next examined if animals retained their seizure susceptibility after being unstimulated for 36 days. Indeed, after pausing stimulation, seizures had higher Racine scores ($p<0.05$, $n=5$) and lasted longer ($p<0.01$, $n=4$) than sessions preceding the stimulation hiatus. The seizure threshold ($p<0.01$, $n=4$) as well as the number of sessions prior to the first seizure was reduced ($p<0.05$, $n=4$). Finally, preliminary immunohistology for NeuN and GFAP indicated that there was no gross neuronal damage nor appreciable glial activation near the injection site.
In summary, our results show that optogenetic stimulation of a small part of neocortex is sufficient to evoke seizure activity in healthy animals. In line with classical kindling findings, we found an elevated retention of seizure susceptibility in kindled animals, a decrease in threshold for seizure activity, and worsening seizure severity, as well as increased seizure duration over time. These results appear to have arisen in the absence of gross brain damage. We anticipate that optogenetic kindling may be incorporated as a new means to selectively examine the contributions of specific cell populations to epileptogenesis.

Disclosures: **E. Cela:** None. **A. Chung:** None. **T. Wang:** None. **P.J. Sjöström:** None.

Poster

592. Epilepsy: Interneurons, Cell Therapies, and Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 592.06/G47

Topic: B.11. Epilepsy

Support: Connecticut Stem Cell Established Investigator Grant

Challenge Award from Citizens United for Research in Epilepsy

NINDS Area #R15NS072879-01A1

Title: Innervation of adult-born dentate granule cells by GABAergic transplants in mice with temporal lobe epilepsy

Authors: *J. GUPTA[1], J. RADELL[1], M. BROMWICH[1], S. GONZALEZ[1], B. W. LUIKART[2], G. AARON[1], J. R. NAEGELE[1];
[1]Biol., Wesleyan Univ., Middletown, CT; [2]Dartmouth Col., Lebanon, NH

Abstract: Medial ganglionic eminence (MGE)-derived GABAergic progenitors transplanted into the dentate gyrus of mice with pilocarpine-induced temporal lobe epilepsy form functional inhibitory synapses onto dentate granule cells (GCs), suppress spontaneous seizures, and improve cognitive impairments (Henderson, Gupta, et al 2014; Hunt et al. 2013; Cunningham et al. 2015). While hippocampal MGE transplants have been shown to synapse with host brain hippocampal neurons, little is known about the neuronal identities of the target cells. Considerable evidence has shown that pilocarpine-induced seizures augment adult neurogenesis in the dentate gyrus, resulting in populations of hyperexcitable, adult-born GCs that hypertrophy, migrate aberrantly, sprout recurrent mossy fibers and form dysmorphic dendrites. In this study, we examined in mice with pilocarpine-induced temporal lobe epilepsy whether hilar transplants of GABAergic interneurons wire selectively with adult-born GCs. Retroviral (pRubi mCherry) labeling was used to identify adult-born GCs at 1, 6 or 12 weeks after status epilepticus. Synaptic inhibition onto these cells by the transplanted interneurons was tested optogenetically in combination with whole cell voltage-clamp electrophysiology and high-resolution images were obtained after biocytin staining. IMARIS 3-D neuron reconstruction software was used to examine the morphology of the adult-born GCs with synaptic connections from the transplants. We found strong transplant-mediated innervation of cells born at 1 week, 6 weeks, and 12 weeks after status epilepticus. The amplitudes of these inhibitory currents correlated with the number of transplant-derived boutons on the recorded granule cells. Innervation by transplant was also associated with changes in dendritic branching pattern as shown by a significant difference in the number of branch points per unit dendritic length (6.32 branch points per 1000 μm dendritic length in cells innervated by transplant vs. 8.15 in cells not innervated by the transplant; $P < .05$). Changes in branching pattern were also revealed by Sholl analysis where neurons that received synaptic innervation from the transplanted interneurons were significantly less branched than non-innervated cells at 20 μm and 30 μm away from their somas ($P < .05$). However, from 120 to 160 microns away from the soma, cells innervated by the transplant were significantly more highly branched than non-innervated cells ($P < .05$). These results indicate the ability of MGE-derived transplanted interneurons to not only provide synaptic inhibition to adult-generated GCs but also lead to alterations in their branching patterns.

Disclosures: J. Gupta: None. **J. Radell:** None. **M. Bromwich:** None. **S. Gonzalez:** None. **B.W. Luikart:** None. **G. Aaron:** None. **J.R. Naegele:** None.

Poster

592. Epilepsy: Interneurons, Cell Therapies, and Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 592.07/G48

Topic: B.11. Epilepsy

Support: MRC

Title: Investigating the neuroprotective mechanisms of potential anti-epileptic drug, canabidiol at specific cell-types in healthy and epileptic models

Authors: *A. KHAN[1], T. SHEKH-AHMAD[2], A. KHALIL[2], M. WALKER[2], A. B. ALI[1];
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Abstract: Non-psychoactive, phytocannabinoids such as Cannabidiol (CBD) are potential anti-epileptic drugs (AED). CBD has given hope for patient groups with uncontrollable epilepsy where other drug treatments have failed, despite the lack of knowledge of the long-term side-effects. To better understand the neuro-protective functions of CBD at identified CNS neurons, we investigated the neuroprotective effects and mechanisms of actions of CBD using in vitro and in vivo models of epilepsy.
Using immunoperoxidase staining the anatomical distribution of two major classes of interneurons, cholecystokinin (CCK) and parvalbumin containing (PV) were determined in healthy and epileptic (post kainic acid) rats.
There was a reduction in the densities of both PV and CCK interneurons, and both cell-types showed distorted morphology in the epileptic model. However, following CBD (100mg/kg) treatment at zero time and 90 mins post status epilepticus, there was a reduction in cell pathology, suggesting that CDB served as a neuro-protective agent. These observations were consistent with electrophysiological whole-cell recordings which showed a decrease in hyper-excitability of principal neurons, whilst selectively enhancing inhibitory interneuron function in the KA and in the Mg2+ free in vitro models of epilepsy.
In summary, the data suggest CBD has a cell-type specific alteration of intrinsic properties of neurons in healthy and epileptic brains. CBD also rescues altered morphological properties of different interneuron subtypes in epileptic brains.

Disclosures: A. Khan: None. **T. Shekh-Ahmad:** None. **A. Khalil:** None. **M. Walker:** None. **A.B. Ali:** None.

Poster

592. Epilepsy: Interneurons, Cell Therapies, and Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 592.08/G49

Topic: B.11. Epilepsy

Support: NIH NS054210

Title: Enhanced output from somatostatin interneurons in malformed cortex

Authors: N. EKANEM[1], N. M. WESTON[1], L. K. REED[1], *K. M. JACOBS[2];
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Abstract: Medically-intractable epilepsies are often associated with cortical malformations. One common form is microgyria, having multiple small folds with underlying irregular lamination in place of normal gyri. Neonatal transcranial freeze lesions in rodents mimic this histopathology and the associated epileptiform activity. Using this model, we have previously shown that in malformed cortex, somatostatin interneurons (SS) fire at increased frequencies in response to depolarizing current and have 3X the normal amount of excitatory synaptic input. Here we bred mice to contain Channelrhodopsin selectively in SS in order to examine the level of output from these neurons. We hypothesize that in malformed cortex hyperactive SS contribute to epileptiform activity via synchronization of columnar activity or via network dis-inhibition through their synaptic contacts onto other inhibitory interneurons. Whole cell patch clamp recordings were made in ex vivo slices from layer V pyramidal neurons adjacent to the microgyrus (paramicrogyral region, PMR) or in homologous control cortex. IPSCs were recorded in isolation with glutamate antagonists APV and DNQX in the bath, a high K^+ intracellular solution, and a voltage clamp holding potential of -70 mV. Blue light was applied through a 60X objective centered over the recorded neuron in order to activate the Channelrhodopsin and depolarize SS interneurons. The duration of the light was varied from 0.1 to 2 msec (11 durations) to create an intensity series. Light-evoked IPSCs recorded from pyramidal neurons had significantly larger peaks in malformed compared to control cortex (2-way ANOVA, $p<0.05$, N = 19 control and 14 PMR neurons). With a duration of 2 msec, the light-evoked IPSC had a peak of -167.5±40.5 for control and -510.5±130.4 pA in PMR. Repetitive light stimulation at 50 Hz produced significantly more depression of the IPSC in malformed, compared to control cortex, suggesting an increased release probability. When the light was moved to layer II/III above the recorded neuron, there was no significant difference in the IPSCs produced in PMR compared to control, suggesting that the increased output from SS in malformed cortex is specifically within layer V. Field potential recordings were also made during light activation of SS with 0.2 mM Gabazine ($GABA_A$ antagonist) in the bathing medium. Under these conditions epileptiform activity was evoked after selective stimulation of SS in the

PMR only and not in control cortex. These results suggest that output from SS is enhanced in malformed cortex and contributes to the production of epileptiform activity. Supported by NIH NS054210.

Disclosures: **N. Ekanem:** None. **N.M. Weston:** None. **L.K. Reed:** None. **K.M. Jacobs:** None.

Poster

592. Epilepsy: Interneurons, Cell Therapies, and Neurogenesis

Location: Halls B-H

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Topic: B.11. Epilepsy

Support: NIH Grant AG049411

NIH Grant AG36800

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NIH Grant AG1049711

DOD Grant PR121769

Evelyn F. McKnight Brain Research Foundation

Title: NSAID treatment reverses age-related changes in hippocampal neurogenesis

Authors: ***J. MCGUINESS**[1,2], R. B. SCHEINERT[2,3], V.-C. SCHWINGEL[2], A. RANI[1], A. KUMAR[1], T. C. FOSTER[1], B. K. ORMEROD[2,1];
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Abstract: Dysregulated inflammatory signaling could contribute to age-related cognitive decline through effects on hippocampal and olfactory bulb neurogenesis. We tested whether non-steroidal anti-inflammatory (NSAID) drug treatment could reverse age-related declines in neurogenesis and spatial behavior in male Fischer 344 rats. Baseline cognitive ability was assessed in young (4-6 mo; n=32), middle-aged (10-12 mo; n=32), and aged (18-20 mo; n=34) rats using a rapid water maze task. In this task, the rats were trained to locate a visible platform in a single session and then a hidden platform in a single session 3 days later. Strength of learning and memory were tested in probe trials administered immediately and 24h after hidden platform trials, respectively. After the first water maze session, the rats were assigned randomly

to vehicle (200µl of frozen strawberry milk; n=9-11 per age group), rosiglitazone (10mg/kg, BID; n=11 per age group), or indomethacin (2.5mg/kg, BID; n=11 per age group) treatment groups. Beginning a week after treatment, rats were injected once daily over 3 days with bromodeoxyuridine (BrdU, 50mg/kg; i.p.) to label dividing cells and were then trained and tested again using the same rapid water maze protocol (without visible platform trials). After the final probe session, a subset of the rats from each NSAID treatment group was perfused to quantify $BrdU^+$ new and IBA-1^+ microglial cell densities and their phenotypes through the hippocampus, rostral migratory stream (RMS) and olfactory bulb. Because neurogenesis measures were similar between middle-aged and aged rats, we combined these groups into an 'aging group' for subsequent analyses. In young rats, indomethacin ($p < 0.05$) but not rosiglitazone increased new cell number relative to vehicle. In aged rats, both indomethacin ($p < 0.05$) and rosiglitazone ($p < 0.05$) increased new cell number relative to vehicle. New cells in the hippocampus correlated positively with new cells in the olfactory bulb GCL ($p < 0.05$). Young rats outperformed aging rats on hidden platform trials (blocks 2-4; p values < 0.05). Both groups performed similarly on the post-treatment immediate probe trial and aging rats actually outperformed young rats on the 24h probe trial ($p < 0.05$). Our results suggest that short-term NSAID treatment can reverse age-related decreases in neurogenesis.

Disclosures: J. McGuiness: None. **R.B. Scheinert:** None. **V. Schwingel:** None. **A. Rani:** None. **A. Kumar:** None. **T.C. Foster:** None. **B.K. Ormerod:** None.

Poster

592. Epilepsy: Interneurons, Cell Therapies, and Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 592.10/H1

Topic: B.11. Epilepsy

Support: DoD Grant PR121769

Title: SST treatment reverses kindling induced changes in adult hippocampal neurogenesis

Authors: *J. A. LEIBOWITZ, G. NATARAJAN, J. ZHOU, M. KING, P. CARNEY, B. ORMEROD;
Univ. of Florida, Gainesville, FL

Abstract: The amygdala kindling model has been used to identify mechanisms and therapeutic strategies for temporal lobe epilepsy (TLE). Hippocampal neural progenitor cell (NPC) proliferation increases 2-to 8-fold and neuronal progeny exhibit aberrant morphologies and connectivity in kindled rats. We have shown that somatostatin (SST) expression driven by AAV5

vector technology blocks epileptogenesis in 70% of rats and have preliminarily shown that SST expression blocks seizure behavior in epileptic rats. To confirm that kindling increases NPC proliferation and neurogenesis, male Sprague Dawley rats (225-250g; n=21) implanted bilaterally with stimulating and recording electrodes into the amygdala were sham or electrically kindled 2x/day to 3 consecutive Racine Grade 5 seizures. They were injected 48h after the final seizure with bromodeoxyuridine (BrdU; 100mg/kg) to label dividing cells and perfused either 4h (n=5-6 per group) or 4w (n=5 per group) later to stereologically estimate total $BrdU^+$ and microglial cell numbers under light microscopy and their phenotypes under confocal microscopy. Significantly more dividing $BrdU^+$ NPCs at 4h ($p < 0.001$) and surviving $BrdU^+$ cells at 4w ($p < 0.05$) were detected in kindled versus sham rats. Kindling upregulated $BrdU^+/GFAP^+/Sox2^+$ Type 1 ($p < 0.05$) but not $BrdU^+/GFAP^-/Sox2^+$ Type 2 NPCs. In these rats, IBA+ microglia number was similar between groups but a greater proportion of $IBA^+/CD11b^+$ microglia ($p < 0.001$) and number of activated ($p < 0.001$) and highly activated ($p < 0.05$) microglia was detected in kindled rats. To test whether SST expression blocked seizure behavior and normalized kindling-induced aberrant neurogenesis, male Sprague Dawley rats were kindled to 3 consecutive Grade 5 seizures and were then injected bilaterally into the CA1 region and dentate gyrus (2µl/site) with pAAV-CBa-GFP control vector (n=5) or pAAV-GFP-SST vector (n=5) to drive SST expression. Three weeks later, rats were given 2-3 test stimulations/week for three weeks, injected with BrdU (100 mg/kg, i.p.) 48h after the final stimulation and perfused 4h later. The average seizure grade was significantly reduced in pAAV-GFP-SST versus pAAV-CBa-GFP rats ($p < 0.05$). Our preliminary data show that pAAV-GFP-SST-treated rats had significantly fewer dividing $BrdU^+$ NPCs than GFP-treated rats ($p < 0.01$) and tended to have fewer Type 1 NPCs ($p = 0.06$) and we are currently quantifying markers of neuroinflammation in these rats. These data support the hypotheses that 1) kindling upregulates Type-1 NPC division, 2) stimulates a neuroinflammatory response and 3) that SST expression may alleviate seizure behavior in epileptic rats by normalizing neurogenesis.

Disclosures: **J.A. Leibowitz:** None. **G. Natarajan:** None. **J. Zhou:** None. **M. King:** None. **P. Carney:** None. **B. Ormerod:** None.

Poster

592. Epilepsy: Interneurons, Cell Therapies, and Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 592.11/H2

Topic: B.11. Epilepsy

Support: DOD Grant PR121769

Title: Persistent somatostatin gene expression treats seizures in a subset of rats with experimental temporal lobe epilepsy

Authors: *G. NATARAJAN[1], J. LEIBOWITZ[2], J. ZHOU[2], M. KING[3], B. ORMEROD[3], P. CARNEY[2];
[1]biomedical sciences, neuroscience, [2]Univ. of Florida, Gainesville, FL; [3]Univ. of florida, Gainesville, FL

Abstract: Sustained adeno-associated viral (AAV) vector-mediated neuropeptide expression is a promising therapeutic strategy for drug-resistant temporal lobe epilepsy. Somatostatin (SST) is expressed widely in the hippocampus and interestingly in hilar GABAergic neurons that are vulnerable to death in temporal lobe epilepsy. We previously demonstrated that sustained SST expression prevents the development of limbic seizures during early epileptogenesis. Here we tested whether sustained SST expression is an anticonvulsant in a rat amygdala-kindling model of temporal lobe epilepsy. Rats were electrically kindled until they exhibited 3 consecutive Racine grade 5 seizures. Subsequently, AAV serotype 5 vector driving GFP (AAV5-CBa-GFP) or rat preprosomatostatin and GFP (AAV5-CBa-SST-GFP) gene expression was injected bilaterally into the hippocampal dentate gyrus and CA1 region. Three weeks later, rats were re-tested at periodic intervals using previously effective seizure-evoking intensities. Kindled rats treated with AAV5-CBa-GFP (n=10) continued to consistently exhibit grade 5 seizures upon repeated retesting whereas 6/13 AAV5-CBa-SST-GFP-treated rats were highly refractory to seizures ($p<0.05$). Rats were persistently seizure free even with prolonged stimulus repetition. These preclinical results suggest that SST may be an alternate therapeutic strategy for pharmacoresistant temporal lobe epilepsy. Kindled rats (n=9) also demonstrated a significant learning impairment on reversal trials on the Morris water maze swim task relative to sham-stimulated rats (n=9). Ongoing studies are directed towards testing whether persistent SST expression in kindled rats (n=10) normalizes the observed reversal trial deficits relative to kindled rats treated with GFP (n=12).

Disclosures: G. Natarajan: None. **J. Leibowitz:** None. **J. Zhou:** None. **M. King:** None. **B. Ormerod:** None. **P. Carney:** None.

Poster

592. Epilepsy: Interneurons, Cell Therapies, and Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 592.12/H3

Topic: B.11. Epilepsy

Support: NIH/NINDS 2R01NS060757

Title: Sonic Hedgehog recues impaired neurogenesis associated with kainic acid epileptic effects

Authors: *L. E. GONZALEZ[1], A. H. KOTTMANN[2], D. M. DURAND[1];
[1]Case Western Reserve Univ., Cleveland, OH; [2]Sophie Davis Sch. of Biomed. Med., CUNY, New York, NY

Abstract: Epilepsy has been associated with dysfunction in hippocampal neurogenesis. In rat neurotoxic models increased neurogenesis in acute and sub-chronic conditions followed by a severe decline has been reported. The time-course from increased to decreased neurogenesis is not generalizable across models. In in the KA mouse model of epilepsy a decline in neurogenesis has been detected to occur as soon as 7 days after the KA injection. Thus, Heinrich et al. (J Neurosci. 2006; 26:4701-13) reported a gradual fall in neurogenesis at 1 week and virtual loss of all neurogenesis by 4-6 weeks after the initial seizure episode. Our data confirm a decrease in neurogenesis on the second week after systemic kainic acid injections in mice. We also found a concomitant decline of Shh transcription, Shh protein and the expression of Shh downstream pathway molecules ptc-1, smoothed (smo) and gli-1 on the second and fourth week after KA injections. As the Shh signaling pathway is a major regulator of neurogenesis, the down-regulation of Shh detected in parallel to declined neurogenesis posed the question of whether selective activation of the Shh pathway could rescue neurogenesis in the KA model. Indeed, we found that the down-regulation of Shh pathway and the decreased neurogenesis could both be rescued by systemic administration of SAG (smoothed agonist). Furthermore, activation of Shh pathway reduced seizures frequency and decreased the number of astrocyte infiltrates. The latter effect suggests that Shh in the SGZ favors neural over astroglial generation. Shh has been found to negatively regulate astrocytes and interruption of Shh signaling in postnatal astrocytes by removal of smoothened resulted in reactive gliosis (Garcia et al. J Neurosci. 2010;30:13597-608). This is significant because astrocytes are the mayor cellular source of inflammatory infiltrates and astrogliosis has been directly implicated in the genesis of epilepsy. Several studies suggest that the reduced neurogenesis found in chronic animal models of epilepsy and human temporal lobe epilepsy, can be associated with persistence of spontaneous recurrent motor seizures, memory impairments and depression. Inducing SGZ neurogenesis using Shh and other trophic proteins has been suggested as therapy in the context of depression and can have potential applications in epilepsy.

Disclosures: L.E. Gonzalez: None. **A.H. Kottmann:** None. **D.M. Durand:** None.

Poster

592. Epilepsy: Interneurons, Cell Therapies, and Neurogenesis

Location: Halls B-H

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Program#/Poster#: 592.13/H4

Topic: B.11. Epilepsy

Support: NIH R00 ES017781

Title: Brain iron loading impairs manganese metabolism and disrupts GABAergic neurotransmission

Authors: *Q. YE, J. CHANG, J. KIM;
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Abstract: Manganese (Mn) is an essential nutrient for neurological function and emerging evidence suggests that Mn deficiency is significantly associated with epilepsy in both humans and animal models. While Mn and iron share metal transporters for absorption and distribution in the body, disrupted iron homeostasis leads to altered Mn transport. In particular, both humans and mouse models with genetic iron overload exhibit reduced blood Mn levels. However, it is unclear whether iron loading impairs Mn metabolism in the brain. To investigate the role of brain iron loading in Mn-associated brain dysfunction, brain samples from the H67D HFE-mutant mice, a mouse model of brain iron loading, and their control wild-type mice (8-10 wk old) were analyzed for Mn-dependent proteins and neurochemical markers related to the epilepsy. First, H67D mice showed elevated brain iron levels by 20% ($p = 0.001$), as determined by inductively coupled plasma mass spectrometry. Notably, H67D mice displayed decreased Mn levels in the blood by 70% ($p = 0.033$) and in the brain by 7% ($p = 0.011$). Moreover, the activity of mitochondrial superoxide dismutase, an essential Mn-dependent antioxidant enzyme maintaining mitochondrial function, was reduced by 13% ($p = 0.026$). In addition, the expression of mitochondrial complexes decreased by 41-52% ($p < 0.050$) in H67D brains. These results demonstrate impaired Mn transport and Mn-dependent cellular function in H67D mice, which could consequently cause neurochemical alterations related to epilepsy. Since decreased GABAergic signaling contributes to the development of epilepsy, we quantified GABA levels in the brain by HPLC. H67D mice displayed reduced GABA levels in the brain by 32% ($p = 0.041$). In addition, H67D mice showed a 178% increase of GABA-A receptor α2 subunits (GAR2 α) as determined by qRT-PCR ($p < 0.001$), consistent with the findings in the mouse model of temporal lobe epilepsy. We found a significantly increased expression of glutamatergic receptor subunits NR2A (55%, $p = 0.021$) and NR2B (177%, $p = 0.003$) receptors in H67D brain, indicating up-regulation of glutamatergic signaling. This is consistent with findings in epileptic patients who exhibit increased expression of NR2 receptors in the brain. Together, our results demonstrate that brain iron loading impairs Mn metabolism and disrupts GABAergic and glutamatergic neurotransmission, which could predispose the development of epilepsy.

Disclosures: **Q. Ye:** None. **J. Chang:** None. **J. Kim:** None.

Poster

592. Epilepsy: Interneurons, Cell Therapies, and Neurogenesis

Location: Halls B-H

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Program#/Poster#: 592.14/H5

Topic: B.11. Epilepsy

Support: NIH R01 NS08056501A1

Title: Early-life seizures alter postnatal development of PV interneurons in the auditory cortex

Authors: *Y. J. SONG[1], E. E. DIEL[2], L. T. MASSARO[1], J. J. LIPPMAN BELL[1], H. SUN[1], T. K. HENSCH[2], F. E. JENSEN[1];
[1]Neurol., Univ. of Pennsylvania, Philadelphia, PA; [2]Mol. and Cell. Biol., Harvard Univ., Cambridge, MA

Abstract: Neonatal seizures are associated with long-term cognitive and behavioral deficits, including autism. Our labs have found that pentylenetetrazol (PTZ)-induced neonatal seizures prematurely unsilence thalamocortical synapses and disrupt critical period plasticity in the auditory cortex (Sun et al., in review), indicative of altered excitatory-inhibitory (E-I) balance. Although the inhibitory GABA circuit is initially underdeveloped at birth, E-I balance coincides with the maturation of the inhibitory parvalbumin (PV) interneurons. One marker of increasing PV cell maturity is their association with perineuronal nets (PNN). These PNN-associated PV cells represent the end of the critical period and an attenuation of plasticity (Hensch, 2005). Here we examine whether early-life PTZ seizures further disrupt E-I balance by altering the postnatal maturational profile of PV cells in the primary auditory cortex (A1).
We induced acute seizures in male C57BL/6J mice by daily i.p. injections of PTZ (60mg/kg) from P9-11, with saline-injected littermates used as controls. Mice were sacrificed for immunohistochemistry at P12, 15, 20 and 40 (control: n = 5, 7, 4, 7; PTZ: n = 5, 4, 6, 6, respectively), and evaluated for the number of PV cells with (PV PNN+) and without PNN (PV+ only) in layer 4 of A1. We observe a developmental increase in the number of PV PNN+ cells within layer 4, with the greatest increase occurring between P12 and 15 in both PTZ and control mice ($p<0.05$, P12 vs P15/20/40). However, no difference in the number of PV+ only or PV PNN+ cells is observed at any age between PTZ and control groups ($p>0.05$), indicating that neonatal PTZ-induced seizures do not affect the number of immature or mature PV cells within A1 layer 4. Interestingly, PTZ treated animals exhibit a trend to increased PV gene expression in homogenized A1 collected during the auditory critical period, measures indicative of either cell number changes in other cortical layers, or molecular changes not affecting cell number. We are currently analyzing cell numbers in each cortical layer to evaluate if the RT-qPCR increase can be attributed to changes in cell number. Our combined results suggest a change in PV cell

maturation in A1 following PTZ-induced seizures. This indicates that early-life seizures can alter local inhibitory networks, which may affect plasticity in areas related to language acquisition.

Disclosures: **Y.J. Song:** None. **E.E. Diel:** None. **L.T. Massaro:** None. **J.J. Lippman Bell:** None. **H. Sun:** None. **T.K. Hensch:** None. **F.E. Jensen:** None.

Poster

592. Epilepsy: Interneurons, Cell Therapies, and Neurogenesis

Location: Halls B-H

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Topic: B.11. Epilepsy

Support: MnDrive Neuromodulation Scholar Funds

NIH Grant R00 NS087110

Title: Axonal sprouting in commissurally projecting parvalbumin-expressing interneurons

Authors: *Z. CHRISTENSON WICK, C. LEINTZ, B. HUANG, C. XAMONTHIENE, E. KROOK-MAGNUSON;
Univ. of Minnesota, Minneapolis, MN

Abstract: Reorganization of hippocampal microcircuitry is a hallmark of temporal lobe epilepsy (TLE), the most common form of epilepsy in adults. A detailed examination of hippocampal reorganization is necessary to understand the progression of the disease as well as to identify new potential therapeutic targets. Previous research has shown that *in vivo* on-demand optogenetic stimulation of inhibitory interneurons expressing parvalbumin (PV) is sufficient to suppress seizures in a mouse model of TLE (Krook-Magnuson *et al.*, 2013). Surprisingly, this intervention was capable of attenuating seizures when PV-expressing interneurons were activated ipsilateral or contralateral to the presumed seizure focus, raising the possibility of commissural inhibition in TLE. There are mixed reports regarding commissural PV interneuron projections in the healthy hippocampus, and it was previously unknown whether these connections, if present, are maintained or modified following the network reorganization associated with TLE. Using retrograde tracer and viral vector labeling techniques, we reveal that healthy control mice do indeed possess a population of commissurally projecting hippocampal PV interneurons. Additionally, we show that commissural PV interneuron projections target several subfields of the contralateral hippocampus. In the intrahippocampal kainate mouse model of TLE we see a slight and not statistically significant decrease in PV interneurons labeled with retrograde tracer from the contralateral hippocampus 2 weeks following kainate injection. This

effect does not last, however, and the percentage of commissurally projecting PV interneurons returns to baseline levels already 1 month following kainate injection. Interestingly, by 6 months post-kainate there is a significant increase in retrogradely labeled PV interneurons, indicating long-range inhibitory axonal sprouting. These findings suggest that PV interneurons supply direct inhibition to the contralateral hippocampus, and to our knowledge, are the first report of such long-range inhibitory axonal sprouting.

Disclosures: Z. Christenson Wick: None. **C. Leintz:** None. **B. Huang:** None. **C. Xamonthiene:** None. **E. Krook-Magnuson:** None.

Poster

592. Epilepsy: Interneurons, Cell Therapies, and Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 592.16/H7

Topic: B.11. Epilepsy

Support: NIMH

HHMI-CURE Medical Student Research Fellowship

Title: Complex, dynamic roles for GABAergic interneurons in initiation and maintenance of optogenetically induced seizures

Authors: *S. KHOSHKHOO[1], V. SOHAL[2];
[2]Psychiatry, [1]Univ. of California, San Francisco, San Francisco, CA

Abstract: GABAergic interneurons play critical roles in seizures, but it remains unknown whether these vary across interneuron subtypes or evolve during seizure initiation, propagation, maintenance, and termination. This uncertainty stems from the unpredictable timing of seizures in most models, which limits neuronal imaging or manipulations around the seizure onset. To overcome these challenges, we developed a mouse model for optogenetic seizure induction. Combining this with calcium imaging, we find that seizure onset rapidly recruits parvalbumin (PV), somatostatin (SOM), and vasoactive intestinal polypeptitde (VIP)-expressing interneurons, whereas excitatory neurons are recruited several seconds later. Optogenetically inhibiting VIP interneurons consistently increased seizure threshold and reduced seizure duration. By contrast, inhibiting DlxI12b-labeled PV+ and SOM+ interneurons reduced both seizure threshold and seizure duration, suggesting that these interneurons suppress seizure initiation, but may help maintain ongoing seizures. These results show how an optogenetically-induced seizure model can be leveraged to pinpoint a new target for seizure control: VIP interneurons.

Disclosures: **S. Khoshkhoo:** None. **V. Sohal:** None.

Poster

592. Epilepsy: Interneurons, Cell Therapies, and Neurogenesis

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Topic: B.11. Epilepsy

Support: NIH RO1 NS082761-02

Title: Post natal manipulation of Arx alters cellular physiological properties and transcriptional profiles in Parvalbumin interneurons

Authors: B. JENNY, *E. MARSH, R. RISBUD, D. JOSEPH;
Div. Child Neurol, Childrens Hosp. of Philadelphia, Philadelphia, PA

Abstract: Introduction: ARX is a transcription factor expressed throughout interneuron development. Mutations in ARX lead to a spectrum of neurodevelopmental conditions with epilepsy as a common feature. Arx is important for interneuron development but continues to be expressed postnatally. We have previously begun to determine the anatomical, behavioral, and physiological implications of post natal Arx loss, but the exact physiological changes and transcriptional alterations are unknown. To address this question, we have utilized a conditional Arx mutant mouse (Arx^{cKO}) that results in loss of Arx in post natal parvalbumin (PV) cells and we performed single cell physiology in cultured Arx^{cKO} and WT cells and are performing RNA-seq on isolated mature PV cells.
Methods: To temporally aboragate Arx from PV cells, we crossed a floxed Arx mouse (Arxfl/fl) with a PV-Cre;tDtomato mouse ($PV^{CRE;Tom}$- mice who express the flourescent reporter tDtomato in PV cells),resulting in complete loss of Arx in PV cells from post-natal day (P)14. For single cell recordings we cultured cortical neurons from P0 ArxcKO and $PV^{CRE;Tom}$ mice. Single cell voltage and current clamp recordings of visually identified pyramidal and non pyramidal cells were performed in a HEPES buffer solution using standard protocols. To isolate PV positive interneurons from adult mice (P35-40), we use the MACs dissociator system (Miltyeni Biotec) followed by FACS sorting of the dissociated cortex. Sorted cell RNA was isolated using Trizol and the purified RNA sequenced using the Illumina miSeq platform. Bioinformatic analysis of the RNA seq data will be performed.
Results: Single cell recordings recapitulated the in situ ArxcKO mice recordings. We found a lower frequency of IPSCs at -40 mV in the pyramidal cKO cells. The Arx^{cKO} mean AHP was lower, Rheobase and action potential amplitude higher for the cKO pyramidal cells. In addition, the firing frequency at 60mV above rheobase was lower for cKO as was the EPSC frequency.

But all did not reach statistical significance at this time. Isolation of PV cells resulted in approximately 30,000-40,000 cells per Arx^{cKO} cortex with low RNA yields. RNA-seq experiment is being performed.
Conclusions: We confirm our previous studies showing alterations in the firing and intrinsic properties of Arx^{cKO} pyramidal cells but in cultured neurons. We also can successfully isolate mature PV cells for RNA sequencing to determine what genes are regulated by Arx in the mature neuron. These data will allow us to hypothesize on downstream targets of Arx that could be implemented as an approach to restoring normal function in patients with Arx mutations.

Disclosures: B. Jenny: None. **E. Marsh:** None. **R. Risbud:** None. **D. Joseph:** None.

Poster

592. Epilepsy: Interneurons, Cell Therapies, and Neurogenesis

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Topic: B.11. Epilepsy

Support: CIHR Grant 8109

CIHR Grant MOP119340

Savoy Studentship

Title: Optogenetic stimulation of principal cells can trigger or dampen ictogenesis.

Authors: *Z. SHIRI[1], F. MANSEAU[2], M. LÉVESQUE[1], S. WILLIAMS[2], M. AVOLI[1]; [1]IPN, McGill Univ. - Montreal Neurolog. Inst., Montreal, QC, Canada; [2]Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada

Abstract: Seizures in temporal lobe epilepsy can be classified as hypersynchronous (HYP) and low-voltage fast (LVF) according to their EEG onset patterns. Experimental evidence suggests that LVF onset seizures mainly result from the synchronous activity of GABA releasing interneurons while HYP onset discharges are contributed by excessive excitatory neurotransmission as well. In this study, we tested this hypothesis using the optogenetic control of calcium/calmodulin-dependent protein kinase II (CaMKII)-positive principal cells in the entorhinal cortex (EC) of transgenic mice, in the *in vitro* 4-aminopyridine (4AP) model. We found that optogenetic stimulation of CaMKII cells using 20 ms light pulses at 2 Hz for 30 s changed both the onset pattern of spontaneous ictal discharges from LVF to HYP discharges and the predominant high frequency oscillations at ictal onset from ripples to fast ripples. Under similar experimental conditions, stimulating these cells using 1 ms pulses at 1 Hz for 180 s

reduced the frequency and duration of spontaneously occurring ictal discharges in some trials, while completely blocking ictal discharges in others. Our data demonstrate the involvement of principal cells in the initiation of HYP onset ictal events as well as the ability of continuous low frequency stimulation of principal cells to disrupt ictogenesis.

Disclosures: Z. Shiri: None. **F. Manseau:** None. **M. Lévesque:** None. **S. Williams:** None. **M. Avoli:** None.

Poster

593. Epilepsy: Networks

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R01DC012545

Title: The feasibility of epileptic seizure prediction using intracranial high frequency connectomics

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Abstract: High frequency oscillations (HFO, 100-500 Hz) in cortical networks have been shown to underlie seizure generation in human intracranial electroencephalographic (iEEG) recordings as well as in in-vitro recordings in various animal models of hippocampal and neocortical epilepsy. HFOs in contrast to other epileptiform discharges such as sharp waves or spikes are the most reliable marker of the epileptogenic area leading to the best surgical outcome when used to define the extent of surgical resections. We assessed the spatiotemporal dynamics of HFOs in pre-ictal, ictal and post-ictal states in contrast to interictal in four patients with intractable epilepsy. Based on band-pass filtered (80-500 Hz) iEEG signals recorded by right fronto-temporo-parietal 8×8 electrode grids, we constructed corresponding functional networks by computing pairwise inter-electrode normalized mutual information (NMI) coefficients for each one-second segment of iEEG data and interpreted the resulting 64×64 NMI matrices as weighted undirected graphs with electrodes representing nodes and NMI coefficients serving as weighted edges. We specifically assessed nodal community dynamics to analyze the temporal evolution of

network topology. Following the concept that seizures can be identified by changes in HFO network community structure, we computed the relative change in HFO partition distance using a sliding window. In all patients, the relative change in the community structure of the interictal state was consistently small, confirming high temporal stability of HFO network partitions during this state. However, network community dynamics started to show critical (> 8%) changes up to 50 s prior to the seizure onset, thus effectively warning against an ensuing seizure. Interestingly, despite widely varied electrographic patterns of cortical activity before the seizure onset, during the electrographic seizure and after seizure termination, the modular dynamics of cortical networks remained remarkably similar. Consequently, a sudden, distinct change in the community structure of the HFO-based dynamic NMI networks was indicative of an imminent epileptic seizure. These findings demonstrate the feasibility of the proposed approach in developing a seizure warning pipeline, based on the concept that electrographic seizures can be characterized by a breakdown of modular structure of HFO networks.

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Poster

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BMBF ERA-Net NEURONII CIPRESS

Title: Mossy fiber synapses in the hippocampal CA2 region in temporal lobe epilepsy

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Abstract: Temporal lobe epilepsy (TLE) is associated with characteristic neuronal loss in the hippocampal CA3 and CA1 regions and in the hilus and with a dispersion of the dentate granule cell layer. These changes induce structural plasticity, in particular sprouting of mossy fibers (MF) into the dentate molecular layer which is associated with recurrent connectivity that has been described decades ago and intensely investigated. We have recently shown that MF sprouting also affects their projection to the CA2 region in a mouse model for TLE (Häussler et al., 2016, Hippocampus): MF sprout into the CA2 pyramidal cell layer and build aberrant somatic synapses on pyramidal cells. Here we aimed at a more detailed characterization of aberrant MF synapses with respect to their expression of synaptic proteins. In addition, we measured epileptic activity in the CA2 region in a higher spatial resolution than previously. To this end, we performed intrahippocampal kainate injections in adult transgenic Thy1-eGFP mice (M-line, Feng et al., 2008, Neuron), which express enhanced GFP mainly in granule cells and MF, and accomplished immunocytochemistry when recurrent epileptic activity and granule cell dispersion have developed. We localized the CA2 region according to the expression of specific proteins (regulator of G-protein signaling 14 (RGS14) or Purkinje-cell protein 4 (PCP4)) and combined these with the synaptic markers synaptoporin, bassoon, vesicular glutamate transporter 1 (vGlut1) and glutamic acid decarboxylase (GAD). Identification and quantification of synapses was performed with Imaris-based 3D reconstruction. In addition, in a subset of mice we implanted high resolution neural probes for *in vivo* recordings into the CA2 region to measure pyramidal cell firing and epileptic activity. We show that MF synapses in the CA2 region of the kainate-injected hippocampus express the synaptic proteins synaptoporin and bassoon which is in favor of a functional synaptic apparatus in the aberrant synapses. Interestingly, they develop a dual phenotype with expression of vGlut1 as well as GAD indicating that intrinsic compensatory processes have taken place that may aim at reducing overexcitation. Nevertheless, CA2 shows strong epileptic population discharges as well as multi-unit firing pointing towards an active contribution to epileptic activity. Support: DFG (grant HA7597), Excellence Cluster ‘BrainLinks-BrainTools’ (DFG-grant EXC1086), BMBF ERA-Net NEURONII CIPRESS

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Poster

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Title: *In vivo* multi-layer calcium imaging of elastic cortical seizure progression

Authors: *M. WENZEL, J. P. HAMM, D. S. PETERKA, R. YUSTE;
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Abstract: Mapping the fine details of epileptic seizure progression is key to understand the pathophysiology of epilepsy. Yet, the in vivo dynamics of seizure progression in densely labeled neural populations have remained unknown. We combine fast in vivo two-photon calcium imaging (30-Hz) with field electrophysiology to map neocortical seizure spread at cellular resolution in a mouse model of acute pharmacological seizures. We find strikingly reliable recruitment of local cortical cell populations, which is appreciable even at the single cell level. Glass microprism assisted multilayer imaging reveals that this reliability holds true across the cortical column. Further, we uncover layer-specific temporal delays during the lateral spread of ictal activity, suggesting an initial supra-granular cortical invasion followed by deep layer recruitment. Intriguingly, despite the same propagation pathways, successive seizures show pronounced compression or expansion in time. We propose an epilepsy circuit model resembling an elastic meshwork wherein ictal progression faithfully follows preexistent neural pathways but can flexibly vary in time.

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Poster

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Title: Role of potassium/chloride co-transporter (KCC2) in 4 aminopyridine (4-AP) induced epileptiform activity

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Abstract: 4-Aminopyridine (4-AP)-induced epileptiform activity is a well-established model of epilepsy. 4-AP increases neuronal excitability by reducing outward voltage-gated potassium currents (e.g., A and D-currents) leading to increased release of transmitters at both inhibitory and excitatory terminals. Recent findings point to the involvement of the potassium/chloride co-transporter (KCC2). In this study, we used 60 channel multielectrode array recordings to characterize spatio-temporal patterns of the epileptiform activity induced by bath application of 4-AP to acute mouse hippocampal brain slices. At low concentrations of bath applied 4-AP (25 – 75uM) we observed interictal spikes characterized by bursting at low frequencies (~1 Hz). At higher concentrations (100 – 200uM) we observed both ictal and interictal events, with ictal events characterized by synchronized activity in the frequency range of 10 – 15 Hz. In all cases, the CA3 subregion of the hippocampus appeared to have the largest activity as compared to CA1 and dentate gyrus (DG). Upon application of the KCC2 antagonist, VU0240551, the higher concentrations of 4-AP were no longer able to generate ictal activity, however the interictal activity remained relatively unchanged at all concentrations. These results are consistent with our past studies of the cortical epileptiform activity and support the hypothesis that in 4-AP conditions the increased release of GABA leading to activation of $GABA_A$ receptors and intracellular Cl^- overload activates KCC2 which then triggers extracellular K^+ increase. This initiates ictal activity. KCC2 works to extrude both Cl^- and K^+ from the intracellular medium, and has been shown to be sensitive to changes in the intracellular Cl^- concentrations. We further explored this hypothesis in a biophysically realistic conductance-based network model including excitatory and inhibitory neurons with dynamic ion (K^+, Na^+, Ca^{2+}, Cl^-) concentrations. In our model, under normal conditions, an increase in intracellular Cl^- for a brief period (5s) in both excitatory and inhibitory neurons resulted in ictal-like activity. In accordance with our hypothesis, reduction of the KCC2 co-transporter prior to the increase in intracellular Cl^- in the model was able to prevent seizures. Overall, we show that (1) development of interictal and ictal activity in the hippocampus is dependent on 4-AP concentration, (2) reduction of KCC2 co-transporter activity prevents the generation of 4-AP induced ictal activity in the hippocampus. Our study provides further evidences for the critical role of the KCC2 co-transporter in the generation 4-AP induced epileptiform synchronization.

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Poster

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Topic: B.11. Epilepsy

Title: Scale free dynamics in human epileptogenic brain tissue

Authors: *S. MOY[1], A. BRAGIN[1], I. FRIED[2], J. ENGEL, Jr.[1], R. STABA[1], S. WEISS[1];
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Abstract: Scale-free dynamics, with a power spectrum following $P \propto f^{-\beta}$ are found in many complex processes in nature. We asked if scale free dynamics were modulated in epileptogenic brain tissue. We analyzed data from 13 patients with mesial temporal or neocortical epilepsy who had been implanted with intracranial clinical depth electrodes for the purpose of localizing the seizure onset zone. We examined the power law relationships of power spectral density for the inter-ictal recordings from each macroelectrode contact in all of our subjects. Overall, 664 out of 780 macroelectrode recordings exhibited a clear power-law relationship in the power spectral density (R-sqaured > 0.85). We found that among these macroelectrode recordings, the slope of the power law (β) was steeper in the non seizure onset zone channels (-3.2291 ± 0.6262) than the seizure onset zone channels (-3.0512 ± 0.6152) (unpaired two-tailed t-test, n = 664, p = 0.0255), indicating that physiological mechanisms specific to the seizure onset zone influence scale-free dynamics. In macroelectrode recordings we also determined the probability distribution of the size of negative deflections in the EEG. While we observed power-law relationships in these negative deflections, β was less than -1.5, suggesting that these areas of the brain were not operating at criticality. We did not observe any difference between the slopes of the seizure and non seizure onset zones in the range of the power law slopes. Future work will determine whether or not certain areas in the epileptic brain function at criticality, and whether or not criticality is found exclusively outside the seizure onset zone.

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Poster

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Topic: B.11. Epilepsy

Support: NIH Grant 5T32GM007055-41

Title: Seizures and sugar, a dietary trigger?

Authors: *K. A. SALVATI[1], P. DAVOUDIAN[1], P. M. KLEIN[1], R. P. GAYKEMA[2], D. R. WYSKIEL[1], M. P. BEENHAKKER[1];
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Abstract: The link between diet and epilepsy remains contentious and the mechanisms through which diet modulates seizures are not well elucidated. Recent evidence suggests that diets leading to low blood-glucose precipitate seizures associated with absence epilepsy. Absence epilepsy is a non-convulsive seizure disorder characterized by a sudden arrest of consciousness and 3-5Hz spike-and-wave discharges (SWDs) in the electroencephalogram (EEG). SWD generation involves thalamocortical circuits comprised of neurons within the reticular thalamic nucleus, primary thalamic nuclei and the cortex. Herein, we investigate how hypoglycemia alters thalamocortical (TC) circuit activity. We first demonstrate in two rodent models of absence epilepsy, the DBA/2J mouse and the WAG/Rij rat, that hypoglycemia increases SWD occurrence (127%, $p<0.001$; 51%, $p = 0.025$) and duration (36%, $p=0.002$; 7.5%, $p=0.18$) after a 16 hour fast. Seizure exacerbation was associated with decreased blood glucose levels (18% drop, $n=10$, $p=0.29$; 28% drop, $n=11$, $p<0.001$) and increased ketone bodies (270% increase, $n=12$, $p<0.001$; 53% increase, $n=6$, $p=0.001$), in mice and rats, respectively. To determine whether ketone bodies, an alternative energy substrate, affect SWD activity, we measured SWD occurrence and duration following injection of insulin. The injection lowered blood glucose (71% decrease, $n=6$) but did not significantly change ketone body levels ($n=6$). Nonetheless, insulin injection increased SWD occurrence (139%, $p=0.043$) in WAG/Rij rats, thus suggesting that SWD modulation occurs specifically via a drop in blood glucose. *In vitro* current- and voltage-clamp recordings suggest hypoglycemic conditions shift TC firing activity from tonic spingle-spike firing to seizure-associated burst mode in response to a low-glucose perfusate. One potential molecular mechanism conferring glucose sensitivity to TC neurons is the ATP-sensitive potassium channel (K_{ATP}). Immunohistochemistry, *in situ* hybridization and qPCR confirm expression of K_{ATP} channels in TC neurons. Moreover, patch-clamp recordings from TC neurons using K_{ATP} channel modulators demonstrate functional K_{ATP}-channel expression in the thalamus. We are currently assessing whether modulation of K_{ATP} channels mediate glucose-dependent absence seizure expression. In sum, we demonstrate that hypoglycemia modulates

thalamocortical circuits likely involved in absence seizure generation. These findings further support the hypothesis that environmental factors, like diet, influence the occurrence of epileptic activity in the brain.

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Poster

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Title: Insult to injury: A new model of epileptiform activity in primate cortex?

Authors: *R. T. BORN[1,2], C. GOMEZ-LABERGE[1], T. S. HARTMANN[1], J. J. NASSI[1,3], A. R. TROTT[1];
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Abstract: Multi-electrode arrays have seen increasing use in both nonhuman primates and humans. One of the most popular devices is the "Utah Electrode Array" (UEA), which consists of a 10-by-10 grid of ~1 mm long electrodes spaced 0.4 mm apart. In order to overcome the surface resistance of the brain, these arrays are typically inserted using a pneumatic piston that applies 15-20 psi of pressure over ~200 ms. This insertion is a traumatic procedure known to be accompanied by microhemorrhages and ultimately to lead to reactive gliosis around the electrodes (Fernandez et al. 2014). In our experience, inserting UEAs into primary visual cortex (V1) of macaque monkeys (n=5) has *never* resulted in abnormal activity, and the responses of the single- and multi-unit activity have been consistent with the abundant literature on V1, including basic receptive field properties, like orientation tuning, and higher order properties such as feature-matched surround suppression (Trott & Born 2015). However, when we performed a second manipulation—the inactivation of feedback from V2/V3 by cortical cooling—we unmasked a latent propensity for epileptiform activity on the UEA in three of three animals. Critically, we have extensive experience (9 monkeys) in which we used the same method of inactivating V2/V3 with "cryoloops" but recorded from multiple early visual areas with single

microelectrodes; in none of these cases did we observe abnormal activity. It was only when we combined UEAs with feedback inactivation that epileptiform activity occurred. In two of the three animals, we recorded normal activity from the V1 UEA for many days *prior* to cooling, but then observed epileptiform activity within minutes of initiating the first cooling session. In these animals, the events terminated shortly following the cessation of cooling, and in all three animals they became less frequent over the course of weeks to months; none ever generalized beyond the confines of the array, and receptive field properties returned to "normal" afterwards. We offer three observations. First, the ability of feedback inactivation to trigger epileptiform activity is consistent with our previous characterization of the role of feedback in surround suppression (Nassi et al. 2013, 2014). Second, the massive data sets acquired from the UEAs for months prior to, immediately before, during and after the initiation of epileptiform activity may prove useful for testing dynamical models of epilepsy (e.g. Truccolo et al. 2011). Finally, our results suggest that the trauma associated with UEA implantation does predispose to abnormal cortical activity that may be revealed by subsequent events that lower cortical inhibition.

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Poster

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Title: How activation of sensory regions can promote propagation of adjacent focal neocortical seizures

Authors: ***S. S. HARRIS**[1], L. BOORMAN[2], A. KENNERLEY[2], P. G. OVERTON[2], Y. ZHENG[3], T. H. SCHWARTZ[4], J. BERWICK[2];
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Abstract: Understanding how seizures propagate across the brain is important to informing ictal symptomatology and improving treatment strategies in pharmacoresistant focal epilepsy. Converging evidence suggests that propagation of focal epileptiform events is restrained by feed-forward inhibition, although the circumstances and processes which result in failure of the

inhibitory restraint remain poorly understood. Previous work by our laboratory and others has indicated sensory adaptation during repetitive stimulation to result in a reduced efficacy of inhibition and attributed this to activity-dependent depression of inhibitory synapses, a mechanism also recently suggested to play a key role in seizure propagation. We therefore hypothesised that the inhibitory restraint which opposes epileptiform discharge propagation might be modulated by local cortical processing to repetitive exogenous stimuli. To test this, we exploited our established multi-modal methodology to examine neural and hemodynamic responses in cortex during physiological stimulation and recurrent focal acute seizures, and our mathematical model that allows decomposition of evoked LFPs into excitatory and inhibitory components. Two multi-channel depth electrodes were implanted into visual and adjacent vibrissal cortex of the urethane-anesthetized rat, the latter loaded with 4-aminopyridine (4-AP, 15mM, 1microliter) for induction of recurrent focal seizures. 4-AP was infused into visual cortex at a depth of 1500micrometers (5min, 0.2microliters/min) after 280s pre-infusion baseline recording. High-resolution spatial measures of oxygenated, de-oxygenated and total haemoglobin concentration were obtained using optical imaging spectroscopy. Whisker stimulation (16s, 5Hz, 1.2mA) was delivered at two different time-points (1000 and 1500s) following 4AP infusion and responses compared to controls to determine the effect of nearby epileptic activity on sensory processing and whether ictal propagation is actively promoted by nearby sensory stimulation. Laterolaminar properties of epileptiform activity and resultant neurovascular coupling processes during the horizontal spread of epileptic activity were concurrently assessed using a suite of analysis techniques. Our findings provide novel evidence that sensory stimulation can promote the propagation of focal acute seizures in adjacent cortex into neighbouring sensory-activated cortex, and indicate complex effects on sensory processing in the ictal penumbra. Our results may have important implications for our understanding of epileptic network properties and the localisation of epileptic foci.

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Poster

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Title: Functional connectivity analysis of interictal neuronal activity

Authors: *K. P. LILLIS, T. JACOB, K. STALEY;
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Abstract: The rapidly developing field of connectomics in neuronal microcircuits can be broadly divided into two categories: anatomical connectomics and functional connectomics. Anatomical synapses can be mapped at a high spatial resolution, but throughput is low and tissue must be fixed. Furthermore, although maps of anatomical synapses can be created with a high degree of certainty, they do not necessarily predict network function: Other factors (e.g. ion channel density) contribute to determining how neurons synchronize and propagate activity. Functional connectivity can be measured in living tissue by analyzing correlated activity in populations of neurons. In previous work, we have demonstrated that functional connectivity changes dynamically with network state. For example, pre-ictal disinhibition unveils scale-free functional network connectivity in seizing brain slices (Lillis et. al. 2015). Thus, functional connectivity does not necessarily directly reflect anatomical connections - rather, it is a level of abstraction for quantifying network output. Here, we used high-sensitivity, red-shifted, genetically encoded calcium indicators (GECIs) to analyze functional connectivity, in spontaneously epileptic organotypic hippocampal slice cultures, during interictal periods of relatively low activity. By imaging sparsely-expressed fluorescent proteins using low-magnification objectives, we were able to record from an entire hippocampal slice culture with single-neuron resolution and single action potential sensitivity. Furthermore, interneurons were labelled by co-expression of green fluorescent protein in DLX-expressing cells. For each of ~500 neurons imaged per recording, an output map was computed by identifying “follower neurons” that activated within 100ms of the index neuron. Organotypic brain slice cultures have no external connectivity and exhibit robust sprouting. Thus computing output maps provides the data to generate input maps for all visualized neurons. Using these data we quantify functional network architecture to look at 1) input maps for interneurons vs principal cells, 2) the relationship between input and output maps, 3) the spatial distribution of functional connections. 4) connectivity vs. age and epilepsy. Preliminary data suggest that, with the higher sensitivity GECIs used in this study, small-world network structure is apparent in “resting state” calcium imaging data. Although this approach identifies connections based on correlation, rather than causation, it represents a stride towards the ultimate goal of functional network mapping in living tissue.

Disclosures: **K.P. Lillis:** None. **T. Jacob:** None. **K. Staley:** None.

Poster

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NIH/NINDS 2R37NS077908-05A1

Title: Computational models of ictogenesis: synaptic depression, recovery, and connectivity

Authors: *T. JACOB, K. STALEY;
Neurol., Massachusetts Gen. Hosp. Dept. of Neurol., Charlestown, MA

Abstract: Objective: The events that transpire as an epileptic network transitions from a non-seizing state to a seizure, i.e. ictogenesis, are enigmatic and very difficult evaluate experimentally. We have created a distributed network model of the CA3 region of the hippocampus that exhibits both interictal spikes and spontaneous seizures. In our simulations, we can record any neuronal or network parameter and study how it changes during ictogenesis. We can then vary parameters of interest to test their influence on ictogenesis. Methods: Our model has a layer of 100 x 100 pyramidal cells, interspersed with a 20 x 20 array of interneurons. The neurons are based on a MacGregor single-compartment model. Every neuron is connected to a neighborhood of surrounding neurons, using several different strategies for synaptic connectivity. Synapses were designed to undergo activity-dependent short-term depression and recovery. The amount of glutamate released at a synapse depends on: 1) probability of release of a glutamate vesicle and 2) the number of releasable glutamate vesicles currently available at the synapse. Both spontaneous release and activity dependent release of glutamate are supported by the model. We also model release of GABA at inhibitory synapses. In this presentation, we report 1) the nature of ictal activity in this network 2) the role that network connectivity plays in seizure generation Results: We found that 1) seizures were comprised of traveling waves whose genesis was strongly dependent on the network distribution of synaptic depression and recovery, and the region within this pattern where ictal activity began 2) small world connectivity increased the probability of ictogenesis, compared to a network connected uniformly. Small world connectivity increased seizure probability by increasing the likelihood of sufficient activity in an ictogenic region of a permissive pattern of synaptic depression and recovery. However, external inputs to uniform networks in ictogenic regions could also generate seizure activity. We are currently investigating scale free connectivity in our network.

Disclosures: **T. Jacob:** None. **K. Staley:** None.

Poster

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Title: Seizing the brain with inhibition and silencing it with excitation

Authors: *L. YEKHLEF[1,2], G. BRESCHI[2], S. TAVERNA[1];
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Abstract: In patients suffering from temporal lobe epilepsy (TLE), the physiopathology of ictogenesis phenomena associated with tonic-clonic seizures is still under extensive investigation. In particular, the role of the different cortical populations is not well understood. We combined optogenetic stimulation and whole-cell patch-clamp recordings in mouse brain slices to dissect the role of glutamatergic neurons and GABAergic interneurons, prior to and during the course of seizure-like events (SLEs) pharmacologically induced in the medial entorhinal cortex. Channelrhodopsin-2 (CHR2) was first expressed under the control of promoters for specific markers of either of the two most common subtypes of GABAergic interneurons, parvalbumin (PV) and somatostatin (SOM). Alternatively, CHR2 was expressed in principal glutamatergic cells (PCs) under the promoter of the synaptic vesicular transporter VGlut2. Direct optogenetic activation of GABAergic interneurons was highly effective in triggering both interictal and ictal discharges which closely replicated analogue events occurring spontaneously. SLEs were associated with a relatively high increase in extracellular potassium concentration and were strongly shortened during extracellular perfusion with the $GABA_A$ receptor antagonist gabazine. Selective PC photostimulation also induced tonic-clonic discharges; however, while interneuronal activation failed to stop or shorten the progression of ictal events, photostimulation of PCs was surprisingly very functional in abolishing ongoing SLEs thanks to a substantial depolarization block effect. Our results suggest that GABAergic interneurons are instrumental in initiating, but ineffective in terminating seizures in the medial entorhinal cortex. Conversely, selective photostimulation of glutamatergic PCs paradoxically blocks the progression of ictal discharges.

Disclosures: **L. Yekhlef:** None. **G. Breschi:** None. **S. Taverna:** None.

Poster

593. Epilepsy: Networks

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 593.12/H21

Topic: B.11. Epilepsy

Title: Optogenetic identification of neural circuits involved in the initiation of nerve agent-induced seizures.

Authors: *J. W. SKOVIRA, D. L. SPRIGGS, J. L. WINKLER, C. E. KAROLENKO, K. M. BOWENS;
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Abstract: Seizures induced by organophosphorus nerve agents, such as soman, are believed to be initiated through overstimulation of the cholinergic system. The substantia innominata (SI) provides the principal cholinergic input to structures known to be highly sensitive to generating seizure activity (piriform cortex and basolateral amygdala). Using classical techniques, such as lesioning or direct injection of drugs, it is difficult to study highly specific neuronal cell types within heterogeneous populations, such as the cholinergic cells of the SI and their projections. Using an optogenetic approach this proof-of-principle study examined whether inhibition by illumination of cholinergic neurons or their projections during seizure onset can modulate the spatial and/or temporal extent of seizure activity. Adult male Long Evans-Tg (ChAT-Cre) rats were surgically prepared 21 days prior to the experiment with cortical screw electrodes to record brain electrocorticograms. An intracranial injection was then made to transduce SI cholinergic neurons using a Cre-inducible recombinant adeno-associated virus vector carrying the Halorhodopsin gene. Optic fiber segments were implanted bilaterally into the SI, piriform cortex, or basolateral amygdala. The indwelling fiber segments were coupled to a DPSS laser (590 nm) during experimentation. Animals received HI-6 (125 mg/kg, IP) 30 min prior to soman exposure (180 µg/kg, SC). Additionally, animals received atropine methyl nitrate (2 mg/kg, IM) at 1min post-exposure followed by 2-PAM (25 mg/kg, IM) + atropine sulfate (0.5 mg/kg, IM) at 5 min after the onset of seizures to reduce toxic signs and increase survival without affecting seizure activity. Following seizure onset cholinergic SI neurons or their projections to the piriform cortex or amygdala were illuminated and electrocortical activity was monitored. Our results show that illumination of cholinergic SI neurons or their projections to sensitive brain areas modulates seizure activity following soman exposure. These results are the first to show that cell type specific manipulation using optogenetics following nerve agent exposure can influence seizure activity and indicate the specific involvement of SI cholinergic neurons early following soman-induced seizures.

Disclosures: **J.W. Skovira:** None. **D.L. Spriggs:** None. **J.L. Winkler:** None. **C.E. Karolenko:** None. **K.M. Bowens:** None.

Poster

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Topic: B.11. Epilepsy

Support: KTIA: NAP_13-1-2013-0001

Title: Multimodal (CCEP and tractography) approach to localize epileptogenic cortex

Authors: ***L. ENTZ**[1], L. HALASZ[1], D. FABO[1], E. TOTH[1], I. ULBERT[2], L. EROSS[1];
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Abstract: Surgery is the most effective therapeutic option for patients with drug resistant focal epilepsy however 40% of operated extratemporal epilepsy patients do not remain seizure free in the long term. In this study, we correlated the results of cortico-cortical evoked potentials (CCEP) mapping with the results derived from probabilistic tractography measurements in order to revel the epileptogenic cortex. Novel non-invasive mapping methods are needed in the future to substitute invasive surgical methods in order to find the epileptogenic cortex.
One patient with refractory epilepsy was implanted with subdural electrodes prior surgical resection. CCEP mapping was performed using bipolar stimulation (10ma, 0.5Hz, 0.2us). Every neighboring electrode pairs were stimulated while the evoked potentials were recorded on the remaining electrodes. An expert neurologist reviewed the ictal EEG to find those electrodes which were involved in seizure onset and early (10 ms) spread. Midpoints between stimulation electrode pairs were selected as seed regions and every recording electrode was selected as a target region for probabilistic tractography. We correlated the zscore of the CCEP amplitudes and mean connectivity values of prob.tractography between the stimulated and the recording electrodes.
We were able to perform probabilistic tractography measurements between desired electrode contacts and established an automated method to run measurements between every stimulated and recorded contact. From 10 stimulations, 5 correlated significantly with the results of tractography using amplitude cross correlation. The significantly correlated stimulations were over ictal areas except one and just one ictal stimulation did not correlate significantly with the tractography. If the CCEPs can reveal effective connections underlying seizure networks, and

prob.tract. shows the white matter projections the combination of these two method can facilitate to survey the expansivity of the ictal network.

Disclosures: **L. Entz:** None. **L. Halasz:** None. **D. Fabo:** None. **E. Toth:** None. **I. Ulbert:** None. **L. Eross:** None.

Poster

593. Epilepsy: Networks

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Program#/Poster#: 593.14/H23

Topic: B.11. Epilepsy

Title: Ventral medial hypothalamus <VMH> and its role in control of spasms.

Authors: *C.-R. CHERN, L. VELISEK;
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Abstract: Ventral Medial Hypothalamus (VMH) is a part of the hypothalamic network and together with the Arcuate Nucleus (ARC) and Lateral Hypothalamus (LH) projects to the Paraventricular Nucleus (PVN; directly or indirectly). PVN in turn regulates the hypothalamo-pituitary-adrenal (HPA) axis. In our previous research, we observed specific seizure phenotype in rats after microinfusion of a $GABA_A$ receptor agonist, muscimol into the PVN. In this project, we determined the role of VMH in the control of spasms by functional activation with bicuculline (a $GABA_A$ receptor antagonist) microinfusion or by functional inhibition with muscimol microinfusion into VMH. We used a rat model of Infantile Spasms (IS). This model uses infant rats prenatally primed with betamethasone, in which spasms are triggered with N-methyl-D-aspartic acid. We evaluated latency to onset of the spasms and the number of spasms as a response to microinfusions of muscimol, bicuculline or vehicle into VMH. There was no significant difference in the latency to onset of spasms or in the number of spasms between control (vehicle-microinfused) and muscimol-microinfused animals. However, we found that with bilateral infusion of $GABA_A$ receptor antagonist, bicuculline, 50% of animals did not develop spasms during the observation time and the remainder experienced a significant delay to onset of fully developed spasms. Our data show that VMH participates in control of experimental spasms indicating a possible role of medial hypothalamus for pathogenesis of infantile spasms. To further investigate these hypothalamic connections we are currently examining an additional structure, the dorsal medial hypothalamic nucleus, which also has direct connection to the PVN.

Disclosures: **C. Chern:** None. **L. Velisek:** None.

Poster

593. Epilepsy: Networks

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Topic: B.11. Epilepsy

Support: KL2TR001432

Research Grant from American Epilepsy Society

Title: Silencing of nigrotectal projections is sufficient to recapitulate the anti-seizure effects of substantia nigra inactivation in diverse experimental models of seizures

Authors: *E. WICKER[1], V. BECK[1], C. SOPER[1], C. KULICK[1], P. N'GOUEMO[2], P. FORCELLI[1,2];
[1]Pharmacol. and Physiol., [2]Interdisciplinary Program in Neurosci., Georgetown Univ., Washington, DC

Abstract: The potent anticonvulsant effect of pharmacological inhibition of the substantia nigra pars reticulara (SNpr), originally identified in the early 1980s by Gale and colleagues, has long been hypothesized to function by disinhibition of the deep and intermediate layers of the superior colliculus (DLSC). The SNpr is a principle source of GABAergic inhibitory input to the DLSC, and consistent with this hypothesis, activation of DLSC is also potently anticonvulsant. Selective targeting of nigrotectal projections (to the exclusion of projections from SNpr to other regions such as thalamus or pedunculopontine nucleus) was not achievable using pharmacological approaches. The advent of optogenetics has enabled direct targeting of this pathway, and thus direct testing of the DLSC disinhibition hypothesis. We have previously reported that optogenetic stimulation of the DLSC is potently anticonvulsant in various models of seizures and epilepsy (Soper et al., 2016). Here, we employed intraperitoneal injection of pentylenetetrazole (which can trigger both forebrain and hindbrain seizures), focal microinjection of bicuculine into the “area tempestas” (to trigger forebrain seizures), intraperitoneal injection of gamma butyrolactone (to trigger absence/spike-and-wave-like seizures) and by auditory stimulation (to trigger generalized tonic-clonic seizures in genetically epilepsy-prone rats (GEPR-3s)). Rats were injected with an AAV coding for the inhibitory opsin, ArchT, into the SNpr and fiber optics were implanted either into SNpr (to silence cell bodies) or within the DLSC (to silence nigrotectal terminals). We found that inhibition of either SNpr cell bodies or the nigrotectal terminals with either unmodulated light or 100Hz pulsed light were effective at reducing seizure severity in all models we examined. Interestingly, the magnitude of seizure suppression was similar between sites of optogenetic silencing. These data show that selective suppression of the nigrotectal pathway is sufficient to account for anticonvulsant effects achieved by inhibition of

the SNpr. The enhanced specificity provided by this approach may further enhance the translational utility and our understanding of this endogenous seizure suppressive circuit.

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Poster

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Topic: B.11. Epilepsy

Support: NIH NS040337

NIH NS044370

Title: Mapping seizure spread during status epilepticus at a single neuron resolution

Authors: *J. KAPUR[1], N. DABROWSKA[2], S. JOSHI[2], J. WILLIAMSON[2];
[1]Dept Neurol., Univ. Virginai Hlth. Sci. Ctr., Charlottesvle, VA; [2]Univ. of Virginia, Charlottesville, VA

Abstract: Seizures propagate from a focus to multiple brain structures, but the neuronal circuits activated during this process are poorly understood. We characterized the temporal changes in the activation of brain regions during prolonged seizures of status epilepticus (SE).
We used TRAP (targeted recombination in activated population of neurons) mice to visualize the brain areas activated during SE. In these mice cfos, which is an immediate early gene that is activated by neuronal activity, regulates the expression of estrogen receptor-tagged Cre recombinase. Administration of 4-hydroxytamoxifen (4OHT) to the mice following a stimulus leads to nuclear translocation of Cre and induction of a reporter protein tdTomato in the population of active neurons. Because the activation of cfos is transient and metabolism of 4OHT is rapid, this strategy allows visualization of active neurons in a restricted window of time (1 hr prior to 4OHT administration). SE was induced by continuous hippocampal stimulation (CHS) for 60 min and cohorts of mice were treated with 4OHT at different times following end of the stimulation. The mice were perfused and brains were processed with passive clarity tissue clearing technique (PACT) 7 days following SE. Images were acquired on a Zeiss 780 confocal microscope.
Mice that underwent electrode implantation only did not show much activation, tdTomato expressing neurons were mostly seen in the ipsilateral hippocampus surrounding the tip of the

electrode. CHS for 60 min induced SE in mice, the continuous seizure activity typically lasted for 120-180 minutes, after which there was low frequency, arrhythmic spike-wave discharges lasting 60-90 minutes. In the mice treated with 4OHT 60 min after the end of stimulation (1st hour of SE), activated neurons were present mostly in the ipsilateral and contralateral hippocampus: dentate gyrus, CA1, CA3 pyramidal layer, lateral entorhinal cortex, olfactory cortex and cingulate gyrus. With the progression of seizures, activated neurons were also detected in the subiculum, lateral entorhinal cortex, olfactory bulbs, and lateral septum in the mice given 4OHT 2 and 3 hr after the end of stimulation, that is during the 2nd and 3rd hour of SE. The number of activated neurons reduced in the 4th hour of SE.
These studies showed that seizures initiate in the hippocampus and there is recruitment of septal and cortical regions with the progression of seizures. In conclusion, TRAP mice combined with brain clarification and advanced microscopy provide a novel tool to map seizure activity at a single cell level.

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Poster

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Topic: B.11. Epilepsy

Title: Disruptions of intrinsic functional connectivity during epileptogenesis in a mouse model of temporal lobe epilepsy

Authors: *H. LEE, S. JUNG, P. LEE, Y. JEONG;
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Abstract: Patients with epilepsy suffer from various cognitive and psychiatric symptoms such as learning and memory impairments, emotional instability, and social behavior deficits. Recent fMRI studies on temporal lobe epilepsy (TLE) reported that alteration of resting-state functional connectivity is associated with these symptoms. One recent study using mice reported that these social behavior deficits and related abnormal cortical activity appeared even in the latent period of epilepsy when epileptogenesis occur. Understanding the changes in the latent period is critical for early diagnosis and management of TLE. In this study, we investigated the alterations of resting-state functional connectivity during the latent period in pilocarpine-induced TLE mouse model using intrinsic optical signal imaging (IOSI). This technique can monitor the changes of local hemoglobin concentration by neuronal activity and can be used for investigating the large-

scale brain intrinsic networks. After seeding on anatomical region of interests (ROI) and calculating the correlation coefficients at each ROI, we built functional correlation matrix and functional connectivity maps in latent and chronic period of epilepsy. We observed significant decreased interhemispheric functional connectivity in frontal, temporal and visual cortex in both periods. We also found that the spectral features of low-frequency oscillations are changed in certain cortical regions in both latent and chronic period. These results show that the even there is no seizure events during the latent period, the change of functional connectivity is ongoing. This finding could help to understand the epileptogenesis and can be used for early biomarker.

Disclosures: H. Lee: None. **S. Jung:** None. **P. Lee:** None. **Y. Jeong:** None.

Poster

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Topic: B.11. Epilepsy

Support: NSF-1264948

Title: Interictal spikes: Hallmark of the ictal onset zone or the epileptic network?

Authors: *E. BAIRD-DANIEL[1], H. MA[1,2], A. G. S. DANIEL[1], M. ZHAO[1,2], J.-Y. LIOU[3], T. H. SCHWARTZ[1,2];
[1]Neurolog. Surgery, Weill Cornell Med. Col., NEW YORK, NY; [2]Brain and Mind Res. Inst. Brain and Mind Res. Inst., New York, NY; [3]Columbia Univ., New York, NY

Abstract: Whether interictal spikes (IIS) arise from the ictal onset zone (IOZ) or the extended epileptic network as well as the etiology and function of IIS are widely debated. Using *in vivo* wide-field bihemispheric calcium imaging we were able to map the onset site of IIS throughout the ipsilateral and contralateral hemisphere in a model of focal neocortical epilepsy. An ictal focus was created via local unilateral injection of 4-aminopyradine (4-AP, 500nl, 15mM) in adult SD rats under isoflurane anesthesia. Ictal events and IIS were recorded through bilateral LFP measurements and simultaneous wide-field calcium imaging. We found that after 4-AP injection, 93.65% of IIS initiated from the contralateral hemisphere (mirror focus). After focal blockade of inhibition with local injection of bicuculline methiodide (BMI, 500nL, 5mM), a GABA antagonist, into the contralateral mirror focus the IIS initiation site switched from the contralateral mirror focus to the 4-AP IOZ, with 91.43% of IIS now occurring ipsilaterally. In a separate group of animals, we injected BMI ipsilaterally ~4mm posterior to the 4-AP injection site, after which we observed that the majority (95.23%) of IIS once again initiated from the

contralateral mirror focus. Consequently, our results suggest that IIS initiation is mediated by GABA-ergic cells in the epileptic network. Suppression of network interactions creates an environment for IIS to arise from the IOZ. This finding suggests that under certain circumstances IIS arise not form the IOZ but the epileptic network, particularly the contralateral hemisphere.

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Poster

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Title: Spatial organization of neural field activity at multiple scales increases during human seizures

Authors: *L.-E. MARTINET[1], G. FIDDYMENT[3], O. J. AHMED[4], E. N. ESKANDAR[2], S. S. CASH[1], M. A. KRAMER[3];
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Abstract: Epilepsy is a complex multiscale disease, extending spatially from microscopic synapses to macroscopic behavioral manifestations. Seizures - the hallmark of epilepsy - exhibit multiscale spatiotemporal dynamics that remain incompletely understood. Here we study the properties of focal seizures using a unique data set of multiscale recordings from microscopic (10x10 grid, 0.4 mm spacing microelectrode array - MEA) and macroscopic (8x8 grid, 10 mm spacing) subdural electrode arrays implanted in patients with pharmacoresistant epilepsy. We find voltage waves propagating along the brain surface in the local field potential (LFP) recorded with the MEA, as well as in the invasive electroencephalogram (ECoG) recordings. To investigate the spatio-temporal coupling between those two scales, we measure the coherence between pairs of electrodes, both within the micro-electrode array and between micro- and

macro-scale. We find that coherence increases during seizure at all scales and that the increase is larger locally at the macro-scale. The group delay between electrodes identifies the presence of planar travelling waves. We find within both the micro- and macro-scale an increased number of waves - and increased consistency of these waves - as seizures generalize. We also show that the direction of propagation is consistent between spatial scales and from seizure to seizure for a given patient. Overall, these results suggest that the spatio-temporal organization of neural populations within and between spatial scales increases during seizure. We hypothesize that this effect may be due to the local circuit activity at the microscopic scale becoming more strongly influenced by macroscopic ictal propagation. We test this hypothesis using a multiscale mean-field model simulating both ECoG and LFP activity. We show that the presence of a slowly diffusing extra-cellular ionic effect can account for a change of spatio-temporal regime during seizure, from a non-organized state to an organized state where activity propagates through consistent waves. A deeper understanding of ictal spatio-temporal dynamics within and between scales may have clinical implications, such as the development of tailored surgical interventions or improved guidance for stimulation induced seizure interruption.

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Poster

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Title: Observations of high-frequency oscillations at human seizure onset from microelectrode array recordings.

Authors: ***J. NADALIN**[1], L.-E. MARTINET[3], G. FIDDYMENT[2], E. N. ESKANDAR[4], C. J. CHU[3], S. S. CASH[3], M. A. KRAMER[1];
[1]Mathematics, [2]Neurosci., Boston Univ., Boston, MA; [3]Neurol., [4]Neurosurg., Massachusetts Gen. Hosp., Boston, MA

Abstract: Human seizures are characterized by rhythmic activity that spans a wide range of frequencies. High frequency oscillations (HFO, >80 Hz) have been widely reported as a spatial and temporal signature of seizure onset. However, many aspects of HFO remain unknown, including the mechanisms that govern HFO, why HFO occurs before a seizure and its causal relation with a subsequent seizure, and whether interventions should target HFO. Here, we examine the features of HFO from microelectrode array (10x10 grid, 0.4 mm spacing) recordings in four human patients with pharmacoresistant epilepsy. We characterize the spatiotemporal dynamics of the HFO, and the evolution of these dynamics during seizure onset. Preliminary results suggest that cross-frequency coupling between fast and slow frequencies occurs, with the temporal localization of high frequency oscillations occurring in the troughs of lower frequency oscillations. Previous work suggests that HFO evolve in patterns of spreading waves of activation, with each wave initiated by activity within a localized cortical region. Here we analyze the spatial organization of the microscopic spatial dynamics of HFO and propagation of activity. A deeper understanding of HFO at human seizure onset promises new insight into the mechanisms of human seizure onset, and possibly treatment.

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Poster

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The Worshipful Company of Pewterers

Title: Differential roles of somatic inhibition in inter-ictal and ictal discharges in a focal cortical seizure model

Authors: *V. MAGLOIRE, J. CORNFORD, D. M. KULLMANN, I. PAVLOV;
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Abstract: GABA(A) receptor blockers trigger seizures while GABAergic drugs are commonly used to treat epilepsy, implicating a failure of the inhibitory system as one of the main causes of epileptic discharges. However, GABAergic interneurons constitute a diverse neuronal population, and the specific contribution of different subtypes of interneurons to the generation and maintenance of epileptic activity remains unclear. Accumulating evidence suggests that GABAergic signalling may, in some circumstances, even facilitate epileptiform activity. Here, we focused on the role of parvalbumin-positive (PV^+) interneurons, which mainly provide fast perisomatic inhibition, in the generation of spontaneous epileptiform discharges *in vivo* in a mouse model of focal cortical seizures. Acute injection of pilocarpine (3 M, 0.2-0.4 μl) into the visual cortex induced either brief inter-ictal bursts (100-500 milliseconds), or ictal-like discharges lasting several seconds. We used closed-loop optogenetic stimulation in combination with a wireless EEG recording system and automated seizure detection to deliver laser-generated light pulses through an optic fibre positioned close to the pilocarpine injection site. Channelrhodospin-2 (ChR2) was selectively expressed in PV^+ interneurons, enabling us to activate PV^+ cells during detected pathological discharges. Optogenetic activation of PV^+ interneurons during periods of inter-ictal like activity facilitated their generation (the last inter-event interval during 10s-long photostimulation was 21 ± 2% shorter than that during the baseline, mean ± SEM, $p<0.01$, n=8 mice) following an initial transient suppression (the first IEI increased by 79±9% compared to baseline, $p<0.05$, n=11 mice). In contrast, ictal-like activity was suppressed by closed-loop photostimulation of PV^+ interneurons. The duration of discharges was reduced by 35 ± 7% (from 3.35 ± 0.57 s to 1.99 ± 0.34 s, n=8, $p<0.05$). To investigate the temporal relationship between somatic inhibition and its ability to attenuate ictal-like activity, we delayed (by 0.5, 2 or >2 s) the delivery of the light pulse after event detection. While photo-activation of PV^+ interneurons 0.5 s into ictal-like events reduced their duration, a delay of 2s and more significantly prolonged the duration of seizure-like discharges (by 34 ± 6%, $p<0.05$, n=5 mice). Thus, we infer that increased activation of perisomatic-targeting interneurons has both pro- and anti-epileptic action depending on the network state. In addition, the use of optogenetic stimulation of PV^+ interneurons to suppress ictal-like discharges is restricted to a narrow time window after the event onset.

Disclosures: **V. Magloire:** None. **J. Cornford:** None. **D.M. Kullmann:** None. **I. Pavlov:** None.

Poster

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Title: Epilepsy-associated mutations of Nedd4-2 in regulating neuronal network activity

Authors: *J. ZHU, K. A. JEWETT, N.-P. TSAI;
Mol. and Integrative Physiol., Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: Epilepsy affects more than 300,000 children under the age of 15 in the United States, according to the Centers for Disease Control and Prevention (CDC), and genetic mutation is an important contributing factor. It is widely accepted that the alterations in activity, composition or distribution of ion channels caused by genetic mutations contribute to the onset of epilepsy. DNA sequencing and analysis performed as part of a worldwide research study called Epilepsy 4000 (Epi4k) recently revealed 329 *de novo* mutations in patients with epilepsy. Among those genes identified, Neural precursor cell Expressed Developmentally Down-regulated 4-like (*Nedd4-2*), was specifically noted. *Nedd4-2* encodes a ubiquitin E3 ligase that has high affinity in ubiquitinating and degrading ion channels. Furthermore, three missense changes in *Nedd4-2* have also been identified in families with epilepsy. Despite these discoveries, the mechanisms by which Nedd4-2 is associated with epileptogenesis are completely unknown. Recently, our lab identified the major subunit (GluA1) of an ionotropic glutamate receptor, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, as a novel substrate of Nedd4-2. Because reduction of the AMPA receptor is known to be critical for leveling down neuronal excitability, and a high level of GluA1 is associated with pediatric epilepsy, our findings led to the *central hypothesis* that Nedd4-2 down-regulates neuronal excitability through ubiquitinating and degrading GluA1. We applied genetic mouse models, electrophysiological and biochemical approaches to investigate the functions of Nedd4-2. With a genetic mouse model, *Nedd4-2*^{andi} mice, in which the predominant form of Nedd4-2 in the brain is deficient, we first found that Nedd4-2 is involved in the regulation of neuronal network excitability. Subsequently our data also indicated that the epilepsy-associated mutations of Nedd4-2 impair its function of degrading GluA1. Our findings reveal the potential link by which Nedd4-2 regulates neuronal excitability. Our continuing study will improve our understanding of 1) protein ubiquitination in neurophysiology, 2) AMPA receptor dynamics and 3) Nedd4-2-associated epilepsies.

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Poster

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Topic: B.11. Epilepsy

Title: Preclinical *In vitro* prediction for the seizure-inducing side effects of drugs

Authors: ***M. GAO**[1], K. SATO[2], Y. IKEGAYA[1];
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Abstract: Epilepsy is a brain disorder marked by recurring seizures. To date, various triggers of seizures have been identified, among which one possible cause is the side effects of drugs. During the preclinical research process of drug development, animal testing is widely used to help screen out the dangerous side effects of drugs. However, it remains hard to predict the seizure-inducing side effects of drugs within this process. Here, we developed an *in vitro* system to monitor the effects of drugs on neuronal activities to detect the seizure-inducing effects. Acute brain slices of mice were applied with drugs in different concentrations, while the local field potential (LFP) of alveus near hippocampal CA1 region being recorded by the 64 channel multi-electrode. Using this system, we tested 12 kinds of drugs that have been reported with seizure-inducing effects to various degrees, including picrotoxin, theophylline, aspirin, enoxacin, oseltamivir, cimetidine, diphenhydramine, ketamine, methamphetamine, diazepam, imipramine and dextran. As a result, drugs reported with high risks of triggering seizures, including picrotoxin, theophylline, enoxacin and diphenhydramine, stably caused abnormal firing in neuronal networks, as was shown by the abrupt changes in amplitudes of LFP, while other drugs showing limited influence. By conducting analysis on the LFP data of different drugs, it was possible to collectively evaluate the risks of seizure-inducing. Therefore, this system seems to be a useful and sensitive tool in the preclinical research process of drug development for seizure-inducing side effect prediction, and might serve as an alternative method of inducing *in vivo* animal testing.

Disclosures: **M. Gao:** None. **K. Sato:** None. **Y. Ikegaya:** None.

Poster

593. Epilepsy: Networks

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Topic: B.11. Epilepsy

Support: Wellcome Trust Clinical Research Fewllowship 106556/Z/14/Z

Wellcome Trust Principle Research Fellowship 088130/Z/09/Z

Title: Dynamic Causal Modelling of GRIN2A-related epileptic abnormalities in sleep

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Abstract: Mutations in the GRIN2A gene - encoding the 2A subunit of the human NMDA receptor - have recently been identified as a major genetic cause for a group of related conditions along the childhood epilepsy-aphasia spectrum. For many patients, language regression and delay, rather than seizures, are the dominant symptoms. However, the EEG of patients within this disease spectrum characteristically shows epileptiform activity with a centro-temporal focus that is strongly enhanced in NREM sleep. This sometimes results in near-continuous epileptiform discharges during slow wave sleep, yet the exact role of sleep and its interaction with NMDAR, remain unknown. According to one hypothesis, epileptiform discharges are hypersynchronous 'perversions' of normal sleep activity, generated by an excitation-inhibition imbalance in the thalamocortical sleep circuit. However, evidence for the hypothesis from human patients has been limited.
Dynamic Causal Modelling (DCM) is an analysis framework that fits generative neuronal models to empirical data. Using Bayesian estimates of model evidence, DCM also allows for direct comparison of competing hypotheses cast in terms of neural models. Recently, it has been applied to identify causative synaptic changes underlying neural signatures of anaesthesia and seizure propagation from EEG. In this study, we apply DCM to invert a network model of relevant thalamic and fronto-temporal nodes for a single patient with a known *GRIN2A* mutation and age-matched controls: Here, cross-spectral densities across sleep stages and epileptiform paroxysms are modelled with coupled neural mass models and differences in the spectral composition of the EEG signal explained with variations in a limited set of model parameters. Inversion over different competing models allows the evaluation of evidence for competing hypotheses regarding the interactions of sleep and seizure mechanism. We specifically tested whether there was evidence for effective connectivity changes between two bilateral perisylvian sources with or without hidden thalamic source.
Through model inversion DCM analysis yields a fully parameterised forward model, explaining the observed changes in EEG output in terms of synaptic changes in a simplified corticothalamic network. This reveals interactions between (i) the sleep-stage specific transitions through parameter space, and (ii) paroxysmal abnormalities underlying ictal discharges. These findings offer a network-level mechanistic description of sleep-related EEG abnormalities in a patient with a known *GRIN2A* mutation, providing a bridge between molecular pathology and network level observations.

Disclosures: M. Heilbron: None. **R.E. Rosch:** None. **K.J. Friston:** None.

Poster

594. Epilepsy: Animal Models

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Topic: B.11. Epilepsy

Support: NIH Grant R01 NS066974

Title: Modulation of thalamic neuronal activity during focal limbic seizures differs by thalamic subnucleus

Authors: *L. FENG[1,2], J. MOTELOW[1], W. BICHE[1], C. MCCAFFERTY[1], N. SMITH[1], M. LIU[1], Q. ZHAN[1], R. JIA[1], A. DUQUE[3], H. BLUMENFELD[1,3,4];
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Abstract: Impaired consciousness in temporal lobe seizures has a major negative impact on quality of life. Previous work has identified the thalamus, particularly the intralaminar nuclei, as a key subcortical arousal region that plays a critical and unique function in regulating arousal, attention and goal-directed behavior. There is also growing evidence that the anterior nucleus of the thalamus (ANT) may have a role in the propagation of seizure activity due to its direct connectivity with the hippocampus. Additionally, relay nuclei such as the ventral posteromedial nucleus of the thalamus (VPM) may play a role in the formation of corticothalamic oscillations. To better understand possible mechanisms and effects in different thalamic subnuclei during focal seizures, we performed separate multiunit recordings in the intralaminar central lateral nucleus of the thalamus (CL), ANT, and VPM in an anesthetized rat model of focal limbic seizures. We found that during focal limbic seizures, the multiunit activity (MUA) in the CL region decreased obviously after seizure initiation, while the ANT demonstrated increasing MUA during the ictal period. In both regions, MUA recordings showed that neuronal firing slowly recovered during the postictal period. In the VPM, the occurrence of spindle waves was markedly increased during seizures. Furthermore, we acquired juxtacellular recordings in the CL thalamus to investigate single neuron activity changes during focal seizures. We found that single neurons in the CL thalamus fired regularly prior to hippocampal seizure initiation, but markedly decreased almost immediately after the seizure began. Firing rates slowly recovered during the postictal period and resumed normal firing after variable intervals. Most neurons in CL fired with a burst pattern during seizures, but fired single spikes during baseline and after recovery. These findings suggest that different subnuclei of the thalamus may play different functional roles during focal seizures, including seizure propagation, cortical inhibition, and formation of slow oscillations. At the cellular level, reduced firing rates and a burst firing mode

for identified CL neurons were recorded during focal limbic seizures. Burst firing has been previously described in thalamic neurons in states such as sleep or deep anesthesia. These results suggest depressed arousal of the CL region of the thalamus, which may suppress the activity of the cortex. Further work is needed to characterize activity patterns in other major thalamic regions. Decreased subcortical arousal could be a critical mechanism for loss of consciousness in focal temporal lobe seizures.

Disclosures: **L. Feng:** None. **J. Motelow:** None. **W. Biche:** None. **C. McCafferty:** None. **N. Smith:** None. **M. Liu:** None. **Q. Zhan:** None. **R. Jia:** None. **A. Duque:** None. **H. Blumenfeld:** None.

Poster

594. Epilepsy: Animal Models

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Topic: B.11. Epilepsy

Support: NIH R01 NS066974

NIH R21 NS083783

Title: Behavioral assessment of the effect of dual-site pontine and thalamic neurostimulation to improve arousal during and after seizures

Authors: M. M. GALARDI[1], ***J. XU**[1], C. P. MCCAFFERTY[1], E. T. MUSONZA[1], J. Y. POK[1], T. R. LIAO[1], A. J. KUNDISHORA[1], A. GUMMADAVELLI[2], J. L. GERRARD[2], M. LAUBACH[4], H. BLUMENFELD[1,2,3];
[1]Neurol., [2]Neurosurg., [3]Neurosci., Yale Univ. Sch. of Med., New Haven, CT; [4]Biol., American Univ., Washington, DC

Abstract: Impaired consciousness during the ictal and postictal states is a major component of morbidity and mortality in epilepsy. The use of deep brain stimulation (DBS) to improve consciousness during and after seizures would represent an alternative therapeutic resource for patients suffering from medically and surgically refractory epilepsy, with a significant impact on their quality of life. To explore this, our lab has developed and uses a rodent model of limbic seizures that reproduces the human electrophysiological and behavioral manifestations associated with loss of consciousness in temporal lobe epilepsy. Some of our recent work has shown that dual-site stimulation of the thalamic intralaminar central lateral nucleus (CL) and pontine nucleus oralis (PnO) applied bilaterally during seizures restored normal-appearing

cortical electrophysiology and markedly improved behavioral arousal in this rodent model. Here we have focused on further investigating the effect of this dual-site stimulation on behavior. We implement an operant behavioral task to assess performance during and after focal seizures and evaluate the cognitive improvements provided by DBS. The task involves responding at an instrumental port (nose-poking) toactivate an adjacent reward port, where rats can collect liquid sucrose rewards when signaled by a stimulus. During successive training sessions, animals show increased percentage of rewards collected, decreased response times, and increased selectivity in responding. After training is completed, we implant electrodes bilaterally in CL and PnO, and unilaterally in the hippocampus and lateral orbitofrontal cortex. Following recovery, animals are reevaluated and, if necessary, retrained in the task until they accomplish optimal baseline performance. Seizures are induced by brief 2 second hippocampal stimulation at 60 Hz in some of the trials within a session. We then stimulate bilateral CL at 100 Hz and PnO at 50 Hz at varying current intensities during and after seizures while synchronously recording electrophysiology and behavior. Task performance was impaired during seizures. Further work is needed to determine the degree of improvement in performance obtained with the dual site stimulation during the ictal and postictal periods. We expect the results that we can obtain through the implementation of our experiments to yield substantial information that will further elucidate the potential benefits of using multi-site DBS to improve consciousness in patients with refractory epilepsy, accelerating the translation of preclinical data into the development of a clinical therapeutic resource.

Disclosures: **M.M. Galardi:** None. **J. Xu:** None. **C.P. McCafferty:** None. **E.T. Musonza:** None. **J.Y. Pok:** None. **T.R. Liao:** None. **A.J. Kundishora:** None. **A. Gummadavelli:** None. **J.L. Gerrard:** None. **M. Laubach:** None. **H. Blumenfeld:** None.

Poster

594. Epilepsy: Animal Models

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Epilepsy Foundation Grant 15-001665

Title: Mechanisms of absence seizures investigated by relating hemodynamics, electrophysiology, and behavioral severity in an awake rodent model

Authors: *C. P. MCCAFFERTY[1], A. KUNDISHORA[1], J. SAMPOGNARO[1], E. JOHNSON[1], W. ISLAM[1], P. VITKOVSKIY[1], P. ANTWI[1], N. SMITH[1], A. MORAWO[1], J. XU[1], M. GALARDI[1], H. BLUMENFELD[1,2,3];
[1]Neurol., [2]Neurobio., [3]Neurosurg., Yale Univ., New Haven, CT

Abstract: Absence seizures, consisting of behavioral arrest and concurrent EEG spike-and-wave discharges, are the most common type of generalized epilepsy. They are the defining symptom of childhood and juvenile absence epilepsy, which are associated with learning difficulties, behavioral disorders and other cognitive impairments. The neural activity underlying absence seizures has yet to be comprehensively characterized, a development that is imperative given the lack of improvement in first-line therapeutics over the past 50 years. Experimental absence seizures have hitherto been studied primarily in anesthetized or neurolept-sedated animals, introducing a difference in arousal state from the clinical condition that may explain discrepancies observed in hemodynamic responses. Consequently, a protocol allowing electrophysiological, hemodynamic and behavioral measurements during unadulterated absence seizures is needed. Further, recent evidence suggesting inter-seizure variation in the degree of behavioral arrest calls for the investigation of arousal and behavioral correlates of consciousness during experimental absence seizures. We developed a head-fixation protocol for Genetic Absence Epilepsy Rats from Strasbourg (GAERS) that minimized stress and allowed expression of seizures. Local Cerebral Blood Flow (CBF) and neuronal activity was thus measured during true absence seizures using combined laser Doppler flowmetry, local field potential and multiunit activity (MUA) recordings. CBF, in multiple cortical regions, increased by ~5% within the first 2 seconds of seizure before decreasing to 95% of pre-seizure baseline over the next ~5s. These changes were accompanied by similar changes to the root-mean-squared amplitude of local MUA in deep layers of the cortex, which initially increased to ~120% of baseline before gradually decreasing to ~90%. These changes are in line with blood oxygen level dependent functional magnetic resonance imaging (BOLD-fMRI) changes in human absence seizures and completely contrast with prolonged increases in CBF, BOLD, and local neuronal activity in sedated animal models. These data strongly suggest that hemodynamics of absence seizures are qualitatively altered by sedation, and therefore that mechanisms of absence seizures should be studied in undrugged models. This head fixation method has been successfully adapted for fMRI, to allow multi-modal assessment of hemodynamics. EEG properties of these seizures will be used to relate hemodynamics and neural dynamics to behavioral correlates of similar events, elucidating the neural and blood flow mechanisms underlying variations in seizure severity.

Disclosures: C.P. McCafferty: None. **A. Kundishora:** None. **J. Sampognaro:** None. **E. Johnson:** None. **W. Islam:** None. **P. Vitkovskiy:** None. **P. Antwi:** None. **N. Smith:** None. **A. Morawo:** None. **J. Xu:** None. **M. Galardi:** None. **H. Blumenfeld:** None.

Poster

594. Epilepsy: Animal Models

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Topic: B.11. Epilepsy

Support: NSF CAREER Award 1149446

Title: The role of respiration and brain alkalosis in adult Naked Mole-Rats seizure

Authors: ***M. ZIONS**[1], T. DZEDZITS[1], D. THEVALINGAM[1], D. P. MCCLOSKEY, 07016[2]; [1]CUNY CSI, Staten Island, NY; [2]CUNY CSI, Staten Island, NJ

Abstract: Adult African naked mole-rats demonstrate robust epileptic seizures under conditions which are likely to induce brain alkalosis, including hyperventilation. The purpose of the current study was to establish appropriate methodology to measure respiration rate in this species, and to determine the alkalosis threshold for hyperexcitability in vitro. We first compared pulse oximetry (Starr Life Sciences), piezoelectric sensors (PVDF wire, Pinnacle EEG), manual counting on slow-motion video, and Eulerian video movement magnification (MIT, CSAIL) to measure respiration rate in freely moving naked mole-rats. The pulse oximetry system reported frequent loss of signal with throat and collar sensors, likely due to the failure of infrared signal penetration through the heavy musculature in the naked mole-rat neck. Better results were obtained with a clip on animals' thighs. Piezo sensors connected to a voltage divider and recorded on an EEG system detected diaphragm movement, but at this time a finer resolution is still needed. High-speed video recorded at 480 frames per second permitted manual counting but with difficulty. Eulerian magnification and visual filtering applied in MATLAB exaggerated ribcage motion and greatly assisted manual counting, and shows promise for measuring laboratory animal respiration if movement is sufficiently restricted. We then recorded epileptiform activity in vitro using extracellular electrodes in naked mole-rat hippocampal slices to manipulate pH. Most slices recorded showed a measurable spontaneous epileptiform burst discharge in area CA3 under routine recording conditions and normal ACSF. When ACSF pH was elevated from 7.4 to ~7.6 through the addition of bicarbonate, bursting frequency increased, indicating a more rapid turnover in the burst-generating mechanism. When carbogen delivery to the slice was removed for 15 minutes and then restored, bursts grew significantly in amplitude and duration, indicating greater recruitment of the excitatory network in burst activity during relative shifts in local pH. Together, these findings suggest that naked mole-rats can provide an excellent model system for understanding the mechanisms of alkalosis-induced hyperexcitability in febrile seizures and similar disorders.

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Poster

594. Epilepsy: Animal Models

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Support: European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement 20 n°602102 (EPITARGET).

Title: Synaptic protein alterations during the acute, sub-acute, and chronic inflammatory response following status epilepticus (SE)

Authors: *U. AVDIC[1,2], M. AHL[2], D. CHUGH[2], I. ALI[2], C. EKDAHL CLEMENTSON[2];
[1]Lund Univ., Malmö, Sweden; [2]Clin. Sci., Div. of Clin. Neurophysiol., Lund, Sweden

Abstract: Synaptic transmission is modulated by synaptic adhesion molecules. Altering the expression of these synaptic proteins may be a new way to "tune" the synaptic activity, both excitatory and inhibitory, and dampen hyperexcitability in epilepsy. The objective of this study was to evaluate the expression of some key synaptic adhesion molecules and synaptic proteins acutely (6h and 24h), sub-acutely (1w), and chronically (4w) following temporal status epilepticus (SE) in rats. We also studied whether protein expression was different in rats that developed spontaneous seizures (epileptogenesis) after SE compared to those with only acute symptomatic seizures until day of perfusion (seizures occurring within 1 week after a brain insult).
Adult male Sprague Dawley rats underwent electrically-induced temporal SE. Electrode-implanted rats with no stimulation served as controls. Animals were continuously video and EEG-monitored. They were sacrificed 6h, 24h, 1w, and 4w post-SE. Brains were removed, sub-divided into cortical, subcortical and hippocampal tissue (ipsi- and contralateral to epileptic focus), and processed for western blot analysis.
In the acute phases (6h and 24h) following SE, we detected a small decrease in the cortex of PSD-95, a scaffolding protein found predominately on excitatory synapses, which may represent an acute reaction to the seizure insult. At 1week, when the vast majority of animals had experienced acute symptomatic seizures, protein changes manifested in the hippocampus with decreased expression of both postsynaptic proteins PSD-95, gephyrin (scaffolding protein found on inhibitory synapses), NL-1 (adhesion molecule in excitatory terminals), and the presynaptic protein synapsin II. This decrease was not sustained 4 weeks post-SE, neither in rats with only acute symptomatic seizures nor in epileptogenic rats that experienced spontaneous recurrent seizures. Instead, we observed 4 weeks post-SE a decrease in the excitatory adhesion molecule N-cadherin in the hippocampus of rats that had become epileptogenic compared to rats with only acute symptomatic seizures.

These results demonstrate synaptic protein changes within the temporal epileptic focus 1 week following temporal SE, at both excitatory and inhibitory synapses, without spread to other cortical/sub-cortical structures. However, these changes were transient. Instead, we found the adhesion molecule N-cadherin to be decreased in the hippocampus specifically in rats that experience spontaneous epileptic seizures, in addition to the initial SE insult 4 weeks post-SE. Further analyses will be needed to verify whether this decrease may predict epileptogenesis.

Disclosures: U. Avdic: None. **M. Ahl:** None. **D. Chugh:** None. **I. Ali:** None. **C. Ekdahl Clementson:** None.

Poster

594. Epilepsy: Animal Models

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Support: NSC 102-2314-B-016 -030 -MY3

TSGH-C104-077

TSGH-C104-079

Title: Levetiracetam prophylaxis ameliorates seizure epileptogenesis after fluid percussion injury

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Abstract: To determine whether post-traumatic seizure severity would be affected by the interval between seizures and head injury, we measured seizures after various times with or without fluid percussion brain injury (2atm fluid percussion injury; FPI). To determine efficacy of anti-seizure medication, we also determined if levetiracetam (LEV) would alter the relationship between injury and subsequent seizures. Early post-traumatic seizures were induced by Kainic acid (KA) at one week after 2atm fluid percussion injury (FPI) in one group (FPI-ES). Seizures were induced at two weeks after FPI by KA in another group (FPI-LS). In addition, one group had induced seizures by KA without FPI, (sham-ES). Finally one group of animals received the antiepileptic agent (levetiracetam) infusion for one week after FPI and then had seizures induced by KA (FPI-LEV-ES). We measured seizure onset time, ictal duration and severity of seizures using a modified Racine's scale. Histopathological changes in the

hippocampus CA1 region were also analyzed. Severity of seizures were increased in the FPI-ES group compared with sham-ES animals. Severity was also enhanced in early post-injury seizures induced by KA (FPI-ES vs. FPI-LS); this exacerbation of seizure severity could be ameliorated by levetiracetam infusion (FPI-ES vs. FPI-LEV-ES). Neuronal degeneration in CA1 was more severe in the FPI-ES group and this degeneration was also diminished by LEV. We conclude that early post injury seizures exacerbate susceptibility and severity of post traumatic seizures and increase neuronal degeneration in the CA1 layer of hippocampus. These changes are partially reversed by LEV infusion after FPI.

Disclosures: Y. Chen: None. **B.J. Hoffer:** None.

Poster

594. Epilepsy: Animal Models

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Topic: B.11. Epilepsy

Support: VA BLR&D BX002305

Title: Effects of glucose and 2DG concentration on epileptiform activity in the hippocampus

Authors: Y. PAN[1], T. SUTULA[1], *P. A. RUTECKI[1,2];
[1]Neurol., Univ. of Wisconsin, Madison, WI; [2]William Middleton Mem. VA Hosp., Madison, WI

Abstract: In vitro brain slice preparations are typically bathed in artificial cerebral spinal fluid (ACSF) with glucose concentrations between 10-20 mM. These concentrations are relatively high compared to the in vivo conditions as lack of cerebral vascular autoregulation in slices requires higher glucose concentration to support diffusion-dependent delivery to achieve physiological concentrations of 2.5-4.4 mM. In rat hippocampal slices, we evaluated the effects of glucose concentration on the rate of epileptiform bursting produced by elevating the extracellular potassium concentration to 7.5 mM and also evaluated the effects of the glucose analogue and glycolytic inhibitor 2D-deoxyglucose (2DG) in suppressing the bursting rate relative to the glucose concentration. Extracellular recordings were made from the CA3 region and the burst frequency was measured in various concentrations of glucose and glucose with added 2DG. Whole-cell voltage clamp recordings from CA3 neurons were made to assess spontaneously occurring EPSCs.
Elevating the glucose to 20 mM from a control level of 10 mM did not alter burst frequency. Also there was no change in lowering the glucose concentration to 5 mM, but 2.5 mM glucose decreased the burst rate by 57%, and 1 mM glucose resulted in cessation of bursting. ACSF with

5 mM glucose and 2.5 mM 2DG decreased the bursting rate 42% with minimal additional reduction in burst frequency produced by 5 mM 2DG (49%). When 2.5 mM 2DG was added to slices bathed in 2.5 mM glucose and 7.5 mM extracellular potassium, the bursting rate decreased by 56%.
2.5 mM glucose decreased the frequency but not amplitude of sEPSCs in control 3.5 mM extracellular potassium, suggesting a presynaptic effect of reducing glycolytic flux. In the presence of 10 mM glucose, 10 mM 2DG has effects on sEPSCs in 7.5 mM but not at 3.5mM extracellular potassium implying that activity is required for 2DG uptake and glycolytic inhibition.
Combinations of reduced glucose and 2DG have synergistic effects in suppressing epileptiform bursts in the hippocampus and demonstrate anticonvulsant actions of reduced glycolytic flux and glycolytic inhibition on epileptic network synchronization that likely contributes to anticonvulsant efficacy of 2DG in multiple acute and chronic rodent models of seizures and epilepsy.

Disclosures: **Y. Pan:** None. **T. Sutula:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); equity in Neurogenomex.. **P.A. Rutecki:** None.

Poster

594. Epilepsy: Animal Models

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Title: Regulation of kindling epileptogenesis by hippocampal toll-like receptors 2

Authors: ***S. MEDEL-MATUS**, E. MINAKOVA, D. SHIN, R. SANKAR, A. MAZARATI; Pediatric Neurol., Univ. of California Los Angeles, Los Angeles, CA

Abstract: Toll-like receptors (TLR) are key components of innate immunity that recognize pathogens and molecules related to cellular dysfunction under conditions of various stressful stimuli, including seizures. Seizures result in increased expression of TLR type 2 (TLR2) and 4 (TLR4) by microglia. While the involvement of TLR types 3 and 4 in ictogenesis and epileptogenesis has been well established, the role of TLR type 2 (TLR2) has been sparsely

explored. The goal of this study was to determine whether TLR2 signaling has modulatory effects on seizures in an animal model of limbic epilepsy-hippocampal rapid kindling. A TLR2 agonist, lipoteicoic acid (LTA, 50 μg, n=8), LTA antibody (LTA-A, 1 μg, n=8) or normal saline (control, 1 μl, n=8) were administered daily over 3 consecutive days, unilaterally into ventral hippocampus of 50 days-old male Wistar rats. Thirty minutes after the last injection, the animals were subjected to kindling (sixty 10-s trains delivered every 5 min; train parameters: 20 Hz, 1 ms pulse duration, square wave monophasic stimuli, 50 μA above afterdischarge threshold [ADT]). ADT and afterdischarge duration (ADD) were gauged before the treatments, 10 min before and 24 hrs after kindling. Kindling progression was analyzed by calculating the number of stimulations required for the development of focal and secondary generalized complex partial seizures. Neither LTA, nor LTA-A affected baseline afterdischarge properties. LTA had no effects on kindling progression. However, LTA-A significantly delayed the occurrence of behavioral seizures (number of stimulations to reach the first behavioral seizure [focal: LTA-A 6.5±1.13, saline 1.62±0.26, $p<0.001$; generalized: LTA 50.25±6.13, saline 25.25±2.67, $p<0.01$]). In addition, LTA-A prevented kindling-induced decrease of ADT (before kindling: 0.76±0.07 mA; after kindling saline: 0.17±0.03 mA; LTA-A: 0.53±0.07 mA, $p<0.001$) and increase in ADD (before kindling: 30.42±1.54 s; after kindling saline 53.63±5.15 s; LTA-A: 26.25±4.38 s, $p<0.001$). The results suggest the involvement of TLR2 in kindling epileptogenesis and kindling-induced increase of seizure susceptibility.

Disclosures: S. Medel-Matus: None. **E. Minakova:** None. **D. Shin:** None. **R. Sankar:** None. **A. Mazarati:** None.

Poster

594. Epilepsy: Animal Models

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Topic: B.11. Epilepsy

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Title: Attenuation of the changes in the expression of BDNF and cfos in adult mice brain undergoing kindling: implications for antiepileptogenic properties of novel antiepileptic compound isoxylitones [E/Z]

Authors: *S. U. SIMJEE[1,2], N. ASHRAF[1], F. SHAHEEN[1], M. I. CHOUDHARY[1,2], A. RAHMAN[1,2], S. U. A. SHAH[1];
[1]HEJ Res. Inst. of Chemistry, Dr. Panjwani Ctr. For Mol. Med., Karachi, Pakistan; [2]Dr. Panjwani Ctr. for Mol. Med. and Drug Res., Karachi, Pakistan

Abstract: Purpose: For the development of new drugs for untreatable epilepsy, it is necessary to clarify the basic pathophysiology involved and find the target site. Immediate early genes such as c-fos followed by expression of brain-derived neurotrophic factor (BDNF) have been evidenced as initial important phenomena in the cascade of molecular systems that develop and complement the neuronal excitation to long-term neuronal plasticity. Both have drawn much attention as a potential therapeutic target for epilepsy. In the present study we have focused on the expression of BDNF and c-fos as a target of novel antiepileptic compound *isoxylitones* [(*E*/*Z* isomeric mixture] in the model of epileptogenesis. **Methods**: Kindling was induced by pentylenetetrazole. Levels of mRNAs and protein for BDNF and c-fos were analyzed in brain samples of mice using RT-PCR and Immunohistochemistry. **Results:** After the generalized grade 5 seizures, induced by chemical kindling, increased expression of BDNF and c-*fos* mRNA and protein was detected in amygdala, cortex, hippocampus and thalamus compared to normal group. In contrast, the treatment of isoxylitones not only retards the development of epileptogenesis but also exhibited a marked reduction in BDNF and c-*fos* levels. Furthermore, the test compound was more effective compared to diazepam since it did not produced neuro- or muscular toxicity. **Conclusion:** The present data suggests that isoxylitones act at the underlying molecular mechanism to control the seizure pattern, such as the suppression of BDNF and c-fos in various key regions of brain. These results further point out the significance of BDNF and c-fos as a target to modify epileptogenic process and to develop antiepileptogenic treatments. Further investigations to explore the mechanism of action of these compounds are under process.

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Poster

594. Epilepsy: Animal Models

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Topic: B.11. Epilepsy

Title: Resected human hippocampal tissue as a platform for testing novel treatments

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Abstract: Epilepsy is a devastating disease and effects about 1% of the general population. Today symptomatic relief is given with anti-epileptic drugs but in 30 % of the patients the drugs

are ineffective. Preclinical research use animal-models to mimic the disease in humans. But due to the complexity of the disease it has proven difficult to translate the findings and new treatments from the animal-models to the human form of the disease. The consequence of this is seen in clinical trials that fail even if the preclinical results looked promising. Some of the drug-resistant patients will undergo surgery to resect the area where the epileptic focus is located. The surgery is usually successful and if the tissue can be resected in one intact piece it can be sliced and the neurons, as well as the neural networks, studied acutely with electrophysiology. The aim for our study was to take advantage of this unique opportunity and create a step in between the preclinical animal-models and the clinic. By creating a platform based on the resected brain tissue from patients with epilepsy to test promising novel treatments and drugs before forwarding them to clinical trials. When the tissue is resected it is carefully moved to our lab, cut into slices and kept in an acute state by interface incubation with oxygenised air above and artificial cerebrospinal fluid (aCSF) below the slice for up to 48h. Electrophysiological patch clamp recordings were performed on individual cells in the granular cell layer in the dentate gyrus at 3h, 24h and 48h time points. To evoke epileptiform activity the slices were perfused with 0 Mg aCSF as well as 0 Mg + 4AP aCSF during electrophysiological recording. Our results show that the intrinsic properties of the cells do not change from 3h to 48h of incubation. We have recorded spontaneous epileptiform activity in normal aCSF as well as 0 Mg aCSF. However this epileptiform activity occur in between long periods of normal activity and is therefore not reliable enough to function as a test-platform. By perfusion of 0 Mg + 4AP aSCF we recorded robust epileptiform activity with a constant occurrence of epileptiform events. By showing that it is possible to keep the slices for up to 48h it enable us to perform more recordings and gather more data in the acute tissue condition. This combined with the robust and constant occurrence of epileptiform events during perfusion of 0 Mg +4AP aCSF is the key for a test platform. By enabling a way of testing novel treatments in human brain tissue before moving on to clinical trails time, money and animals can be saved. The resources can be directed into the treatments that actually function in the human brain and the ones that do not can be sorted out in an early stage.

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Poster

594. Epilepsy: Animal Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 594.11/J6

Topic: B.11. Epilepsy

Title: Comparative study in two rodent species of the efficacy of anticonvulsant substances in the 6 hz psychomotor seizure test

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Abstract: The 6-Hz test was originally described as a model of psychomotor seizure characterized by differing sensitivity to Phenytoin and Levetiracetam. Usually performed in the mouse, this screening test is recommended for identifying potential new anticonvulsant substances against partial seizures. The aim of the present study was to evaluate the utility of adapting this test in the rat. We first compared the effects of increasing current intensities for inducing seizures in the mouse and in the rat, followed by the evaluation of the pharmacological profile of anticonvulsant substances in both species. Mice or rats were manually restrained and an electrical stimulation was administered via corneal electrodes connected to a constant current shock generator. The seizure was characterized by the presence or absence of forelimb clonus immediately after electrical stimulation. Following the evaluation of their anticonvulsant effects, the possible adverse effects of drugs were evaluated by measuring locomotion. In the rat, the forelimb seizure score was intensity-dependently increased and seizures were observed in all the animals tested at 44 mA. The seizures were of lower magnitude in the mouse and they were not observed in all mice stimulated at 44 mA. Levetiracetam clearly decreased the forelimb seizure score over the dose-range 100-300 mg/kg in both species without affecting locomotor activity. Valproate displayed anticonvulsant activity at 200 mg/kg and fully protected both species at 300 mg/kg, a dose producing sedative effects in the mouse. Lamotrigine partially antagonized forelimb seizure from 10 to 60 mg/kg in the mouse and from 30 to 60 mg/kg in the rat, but induced clear motor impairments at the high dose in both species. Behavioral toxicity characterized by seizures was observed with lamotrigine at 100 mg/kg in the mouse. Phenytoin showed slight anticonvulsant activity at 10 mg/kg in the mouse and 30 mg/kg in the rat but the effects were not confirmed at higher doses. Although the magnitude of seizures was differed in the mouse and the rat for a given current intensity, the pharmacological profile of anticonvulsant substances in the 6 Hz test was very close in both species. Dose-dependent efficacy of Levetiracetam and Valproate was observed in the absence of clear efficacy of Phenytoin. Lamotrigine also showed anticonvuslant activity, even if the effective dose was close to the toxic dose. The 6 Hz test in the rat can therefore be useful as a screening model prior to follow-up models of partial seizures usually performed in the rat, such as the amygdala kindling or the pilocapine model of spontaneous recurrent seizures.

Disclosures: **E. Esneault:** None. **G. Peyon:** None. **E. Sablé:** None. **V. Castagné:** None.

Poster

594. Epilepsy: Animal Models

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Topic: B.11. Epilepsy

Support: NRW-Rückkehrerprogramm

Human Frontiers Science Program

DFG SPP1757

DFG SFB1089

Title: Rapid astrocyte morphology changes support epileptic activity

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Abstract: Astrocytes actively contribute to the functioning of neuronal networks. Their close contact to thousands of neurons enables them to modulate and maintain neuronal function effectively by, for example, buffering potassium and glutamate clearance. A disruption of this spatial relationship could be of pathophysiological significance. Indeed, astrocyte dysfunction and long-term morphology changes have been implicated in numerous diseases including epilepsy. How rapid astrocyte morphology is altered by the onset of epileptiform activity and to what degree this contributes to aberrant network activity is largely unknown. Combining established protocols of hippocampal epileptogenesis, electrophysiology and two-photon excitation fluorescence microscopy allowed us to monitor astrocyte morphology changes during the induction of epileptiform activity in acute rodent hippocampal slices. Analysis revealed that small and medium-sized astrocyte processes shrink acutely within minutes after epileptiform discharges appeared in the CA1 region. Importantly, similar astrocyte morphology changes were also detected 30 minutes after induction of status epilepticus *in vivo* by intracerebral kainate injection. *In vitro*, these astrocyte morphology changes outlasted the induction of epileptiform activity, persisted after pharmacological termination of epileptic activity by TTX and were sensitive to inhibition of Rho-associated protein kinase (ROCK, Y-27632). Interestingly, ROCK inhibition also reduced epileptiform activity, indicating that rapid astrocyte morphology changes support epileptic activity. A modification of glutamatergic or GABAergic synaptic transmission

did not underlie the proconvulsive effect of astrocyte morphology changes. Instead, we observed that intracellular diffusion in astrocytes and diffusion between astrocytes via gap junctions were significantly decreased in parallel to morphology changes. The reduced astrocyte gap junction coupling is likely a consequence of reduced intracellular diffusion because no change of connexin 43 and 30 expression and phosphorylation was observed. Thus, astrocytes respond to epileptic activity with morphology changes on a time scale of minutes, which reduces intra- and intercellular diffusion in the astrocyte network and supports further epileptic activity. Current experiments investigate the mechanism (e.g. potassium buffering and glutamate uptake) linking acute astrocyte morphology changes and the maintenance of epileptiform activity.

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Poster

594. Epilepsy: Animal Models

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Program#/Poster#: 594.13/J8

Topic: B.11. Epilepsy

Title: Association of ELAVL2 and PTPRD genes with epilepsy in purebred Chinese Crested Dogs

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Abstract: We performed a genome-wide case-control study in a sample of epileptic Chinese Crested Dogs and matched controls to investigate the contribution of genetic variants to the high epilepsy risk in the breed. The genotyping was performed using the Canine HD Whole-Genome Genotyping BeadChip (Illumina). After quality controls, we obtained a dataset of 27 cases and 38 controls (Call Rate > 80%) for a total of 106,392 informative autosomal SNPs. Association analysis at the SNP level was conducted using PLINK running a logistic regression including age, gender and correcting for population stratification. Quality of candidate SNPs detected was further assessed by visually inspecting the genotype cluster plots. None of the SNPs analyzed reached genome-wide significance (P = 4.7E-07). However, at nominal significance levels we observed 7, 15 and 2 SNPs with P < 0.001 in the additive, dominant and recessive models, respectively. Among the top genes we observed KHRDBS3, ELAVL2, TCF25, DEF8, PTPRD, RNF25, GALNT10, DPYD, ATAD2 and WDYHV1. The association test was rerun for the

SNPs located in the most potentially biologically relevant genes for epilepsy (KHRDBS3, ELAVL2 and PTPRD) with additional samples not included in the GWAS due to call rate, for a total of 4 or up to 9 additional samples depending on the SNP. The results confirmed the significance for the SNPs located in PTPRD and ELAVL2 (P = 9.9E-05 and P = 9.9E-05, respectively). The results for the variant located in KHRDBS3 were not considered due to the low quality of the additional samples for that particular SNP. Our preliminary results suggest the involvement of variants located in ELAVL2 and PTPRD with epilepsy in the Chinese Crested Dog breed. ELAVL2 is highly expressed in hippocampal CA3 pyramidal neurons and hilar interneurons. It has also been reported in animal models that Elavl2 protein level in the hippocampus is acutely down regulated after a kainic acid induced seizure in the hippocampal CA3 region. PTPRD was detected suggestively significant in a GWAS investigating the association with remission of seizures after starting treatment in 889 newly treated patients. We also observed 2 SNPs located in ADAM23 (P = 0.0012), a gene previously associated with idiopathic epilepsy in 4 different dogs breeds. Due to our limited sample size, the results are not significant at the genome-wide level after correction for multiple testing. A larger sample size will help to confirm the results; however, the biological significance of some of the top genes justifies a potential role of these variants in epilepsy in the Chinese Crested Dog.

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Poster

594. Epilepsy: Animal Models

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Topic: B.11. Epilepsy

Support: Newcastle University FMS Translational Research Fund Award

Wellcome Trust/EPSRC Innovative Engineering for Health Award 'CANDO'

Title: Tumor-associated epilepsy in a mouse model of malignant glioma.

Authors: *E. STOLL[1], C. GANDARA DE SOUZA[2], V. AFFLECK[2], J. STOCKTON[3]; [2]Inst. of Neurosci., [3]Undergraduate Programme in Biomed. Sci., [1]Newcastle Univ., Newcastle Upon Tyne, United Kingdom

Abstract: Glioma is the most common form of adult-onset primary malignant brain tumor, affecting approximately 4 per 100,000 people and representing 81% of all malignant brain

tumors. Low-grade gliomas commonly manifest with a seizure, in approximately 75% of cases; high-grade gliomas, called glioblastomas, are associated with seizures in 29-49% of cases (Ostrom *Neuro Oncology* 2014, CBTRUS *Statistical Report* 2009). Tumor-associated epilepsy (TAE) significantly impacts on the quality of life of these patients.

Initiating events underlying TAE are not well-understood, but are thought to include mass effect during tumor growth, with attendant increases in intracranial pressure; loss of interneuron populations; aberrant potassium ion channel expression within neurons, leading to depolarizing not hyperpolarizing responses to GABA signals; excess glutamate release by the tumor or altered responses of cells to this neurotransmitter; alterations in gap junctions between cells; altered cation buffering by glial cells or altered glial excitability; pH shifts due to the acidic environment created by tumour cells; or localised disruption of neural networks near the tumor (Cowie & Cunningham *Epilepsy & Behavior* 2014).

An animal model of malignant glioma which demonstrates tumor-associated epilepsy may help to elucidate the underlying mechanisms for this phenomenon, although few characterised models are available (Kirschstein & Kohling *J Neurosci Methods* 2015). Recently, a syngeneic mouse model of glioma has been developed which reproduces the histopathological and clinical characteristics of human glioma on a wild-type background (Mikheev et al. *Aging Cell* 2009). We observe that 26% of these animals manifest seizures, thereby providing a highly reproducible context in which to evaluate mechanisms underlying TAE.

In this study, we conducted histological analysis in seizing and non-seizing animals to discern cellular and molecular features associated with this phenotype. Animals with seizures do not have significantly larger tumor size or greater infiltration of brain areas, nor do they demonstrate significant changes to interneuron population size in the ipsilateral peri-tumoral region, although some cellular disorganisation is observed. Future studies will address mechanisms underlying TAE by using Utah Arrays to record single-unit activity and field potentials across tissue sections containing both tumoral and peri-tumoral regions, while pharmacologically manipulating glutamate, GABA, and gap junction signalling.

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Poster

594. Epilepsy: Animal Models

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Topic: B.11. Epilepsy

Support: EC - FP7 - Health-F2-602531-2013

Title: A model of bilateral subcortical band heterotopia created by *In utero* electroporation with a triple electrode probe

Authors: *S. SAHU[1,2], E. BUHLER[3,2], Y. CHAUVIN[1,2], A. REPRESA[1,2], J.-B. MANENT[1,2]; [1]INMED, INSERM U901, Marseille, France; [2]Aix Marseille Univ., Marseille, France; [3]INMED, PPGI, Marseille, France

Abstract: Experimental subcortical band heterotopia (SBH) can be modeled in rats by knocking down Dcx in embryonic brains using in utero electroporation and RNA interference. These Dcx-KD rats display altered neocortical excitability, resulting in an increased propensity for convulsant induced seizures at juvenile ages and spontaneous focal, absence-like seizures in adulthood. These rats however harbor a band heterotopia located in a single hemisphere (unilateral), unlike most human patients harboring bilateral and symmetrical SBH. Also, spontaneous seizures were rarely observed before 5-6 months of age in unilateral Dcx-KD rats, while epilepsy usually begins between childhood and infancy in most patients with SBH. To overcome these limitations and increase the strength and relevance of experimental SBH regarding the human situation, we recently introduced a novel rat model with bilateral SBH. This rat model is generated via in utero electroporation by utilizing a triple electrode configuration enabling simultaneous electroporation of the two brain hemispheres (bilateral) instead of a single hemisphere in the conventional settings. Histological analyses revealed the presence of widespread band heterotopia in both hemispheres of Dcx-KD rat brains. Telemetric EEG recording at different age (1-6 months) showed that rats with bilateral SBH develop spontaneous recurrent spike-and-wave discharges at early ages. Furthermore, whole cell voltage clamp recordings on acute juvenile neocortical slices revealed an increased ongoing glutamatergic synaptic activity and the occurrence of spontaneous network driven activities in the cortex overlying SBH. Our preliminary results indicate that this novel model could be useful to study the mechanisms leading to spontaneous epilepsy in an experimental context more relevant to the clinical situation seen in humans.

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Poster

594. Epilepsy: Animal Models

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Title: Interneuronopathy in a non-genetic animal model of infantile spasms

Authors: *A.-M. KATSAROU[1], S. L. MOSHÉ[1,2,3], A. S. GALANOPOULOU[1,2];
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Abstract: **Background:** West syndrome is an age-specific type of severe epilepsy of infancy, which manifests with characteristic seizures, infantile spasms (IS), and poor prognosis. Numerous structural, metabolic or genetic etiologies have been linked to IS. Interneuronopathy, a dysfunction or abnormal migration of interneurons, has been associated with certain genetic etiologies of IS. We have developed a non-genetic animal model, the multiple-hit rat model of infantile spasms due to an acquired structural lesion, to investigate further the pathogenesis of West syndrome.
Objective: Here, we investigate if interneuronopathy is a feature of IS of acquired etiologies. We focused on parvalbumin-immunoreactive (PRV-ir) GABAergic interneurons, which comprise almost half of the GABAergic cortical interneurons, and investigated whether they are selectively reduced in the cerebral cortex of multiple-hit rats with IS.
Methods: Postnatal day (PN) 3 Sprague-Dawley male rats received right intracerebral injections of Doxorubicin and Lipopolysaccharide followed by systemic p-chlorophenylalanine on PN5 (herein called DLP rats). On PN20, rats were subjected to transcardiac perfusion and brains were frozen for histology. PN20 male control rats were also used. Coronal brain cryosections (40μm) were stained with a primary mouse anti-PRV antibody (SIGMA, St Louis, MO). PRV-ir cells (cells/mm^3) from all layers of the left or right sensory (S1, S2) cortex and cortical volumes were counted in 8 DLP and 7 control rats, blinded to treatment allocation. Median test was used for statistical comparisons.
Results: In the left sensory cortex, cortical thickness of DLP rats was reduced only to 91.8% of controls (P=0.0553), yet there was a preferential reduction in the densities of PRV-ir interneurons in DLP rats (median=1006.94 cells/mm^3) compared to controls (median=1973.27 cells/mm^3) (P=0.0062). In the right sensory cortex of DLP rats, there was a significant reduction in cortical thickness (57.2% of controls, P=0.01) but no further selective reduction in PRV-ir cell densities.
Discussion: In the right sensory cortex, the induced lesion is not cell-type specific, manifesting as severe cortical thinning. In the contralateral cortex, however, there is preferential loss of PRV-

ir interneurons. Interneuronopathy can be a feature of certain acquired etiologies of IS extending beyond the borders of the primary structural lesion.

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Poster

594. Epilepsy: Animal Models

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Topic: B.11. Epilepsy

Support: SFB 1089

Title: Novel chemo-optogenetic model of inducible epileptic seizures

Authors: ***R. MITELMAN**[1], D. R. LEVY[1], L. POTHMANN[2], H. BECK[2], I. LAMPL[1], O. YIZHAR[1];
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Abstract: Epilepsy, one of the most common and devastating neurological disorders, is manifested by synchronized neuronal seizures that typically begin as a local-circuit phenomenon and rapidly spread to remote brain regions, impairing cognition, motor functions and sensory perception. Better understanding of the pathophysiology of seizure onset, propagation and changes in excitation/inhibition (E/I) balance is required for the development of new therapeutic approaches. However, current animal models suffer major weaknesses rendering them less suitable for these purposes. Reliable models of epilepsy (e.g., pilocarpine), lack a method for seizure induction, whereas reliable seizure models (e.g., PTZ induced seizure), require radical interventions in the network activity. We have developed a novel chemo-optogenetic model that allows light inducible seizures, with high reproducibility at a defined cortical locus. In this model we used AAV stereotactic injections to express an inhibitory chemogenetic tool (hM4D) along with channelrhodopsin-2 in GABAergic interneurons of the medial prefrontal cortex. Application of the synthetic ligand of hM4D Clozapine N-oxide (CNO) silenced GABAergic neurons, as we demonstrated by acute brain slice recordings. Importantly, systemic application of CNO in non-anesthetized mice initiated a global seizure within a few minutes, as expected by silencing the inhibitory population of neurons. Paradoxically, when the same population of inhibitory cells was optogenetically activated days later, prolonged epileptic activity also developed, lasting minutes after the light was terminated. We also observed spontaneous epileptic activity in some of the animals. Strikingly, systemic pre-treatment of these mice with

Bumetanide, a blocker of NKCC1 transporters, attenuated the optogenetically-induces seizures. NKCC1 pumps chloride into the cells and is not expressed in healthy adult neurons. Therefore, our results suggest that seizure-associated re-expression of NKCC1 in neurons raises GABA reversal potential, making these synapses less inhibitory or even excitatory. In summary, this novel model combines the advantages of both a chronic epilepsy model, as the animals show spontaneous epileptic activity, and an acute seizure model, with inducibility by optogenetic activation of GABAergic neurons. Seizures are likely to result from a shift of GABA reversal potential, rendering the network more excitable. This inducible model allows highly controlled testing of the underlying cellular and network mechanisms of seizures, such as changes in the E/I balance and their spatiotemporal propagation pattern.

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Poster

594. Epilepsy: Animal Models

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Topic: B.11. Epilepsy

Support: CONACYT SCHOLARSHIP EVC 326059

Title: Status epilepticus induced by pentylenetetrazol and lithium-pilocarpine increases cell proliferation in the developing rat cerebellum

Authors: *E. VELAZCO[1], I. ZAMORA BELLO[2], A. A. PUIG LAGUNES[2], L. BELTRAN PARRAZAL[2], C. A. PEREZ ESTUDILLO[2], C. MORGADO VALLE[2], M.-L. LOPEZ MERAZ[2]; [1]Ctr. De Investigaciones Cerbrales, Xalapa, Mexico; [2]CENTRO DE INVESTIGACIONES CEREBRALES, UNIVERSIDAD VERACRUZANA, XALAPA, Mexico

Abstract: Evidence supports that *status epilepticus* (SE) promotes neuronal proliferation and differentiation in the adult and developing rodent hippocampus. However, the effect of SE on other neurogenic brain regions, such as the cerebellum, has been less explored. The goal of this research was to determine whether SE induced by pentylentetrazole (PTZ) and lithium-pilocarpine (Li-Pilo) increases cell proliferation in the developing rat cerebellum. SE was induced in fourteen-days-old (P14) Wistar rat pups (both sexes). PTZ-induced SE was produced by injection of 55 mg/kg (n=6); Li-Pilo SE was induced by given 3 mEq/kg lithium chloride on the day before the induction of SE with 100 mg/kg pilocarpine hydrochloride. Control animals were given an equal volume of saline or lithium chloride followed by saline, respectively. One h

after SE and the following day, rats were injected intraperitoneally with 5-bromo-2-deoxyuridine (BrdU, 50mg/kg). Seven days following SE, rats were anesthetized and transcardially perfused with 4% phosphate-buffered paraformaldehyde; control rats were processed similarly. Subsequently, the cerebellum was removed and cut (40-µm-thick sagittal sections) in order to perform peroxidative immunohistochemistry to detect BrdU in the cerebellar vermis. This protocol allowed to quantify the number of cells that incorporated BrdU (BrdU+), indicative of cell proliferation, in the granular layer of the vermis. Data were analyzed by a Student *t* test. Results showed a significant increase in the number of cerebellar BrdU+ cells after SE induced by PTZ (121 ± 5.5) or Li-Pilo (98 ± 4) when compared with the control group (77 ± 3.4 and 70 ± 3.2, respectively). PTZ-induced SE increased the number of BrdU+ cells specifically in the lobules II (81.5 ± 10.5), III (122 ± 17.5), VIb (165 ± 17.2), VIc (161 ± 24.8), VIII (179 ± 14.4), IXa (108 ± 10) and IXb (176 ± 14.3) when compared with the control group (49 ± 7.8, 73 ± 9.4, 80 ± 15.4, 11.5 ± 96, 93 ± 8.2, 11.9 ± 73 and 82 ± 10.4 respectively). Li-Pilo-induced SE increased the number of BrdU+ cells in the lobules II (67 ± 4.3), V (100 ± 11.3), VIc (151 ± 13.5), VII (130 ± 11.1) and X (87 ± 10.1) compared with the control group (44 ± 8.5, 67 ± 7.3, 14.7 ± 86, 92 ± 8.2 and 56 ± 7, respectively). In conclusion, SE induced in the developing rat by different experimental models increases cell proliferation in the granular layer of the cerebellar vermis.

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Poster

594. Epilepsy: Animal Models

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Topic: B.11. Epilepsy

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Title: Interictal and ictal involvement of the anterior nucleus of the thalamus in a non-human primate model of mesial temporal lobe epilepsy

Authors: A. SHERDIL, Jr[1,3], S. CHABARDES[2], A. DAVIN, Jr[2,3], S. MICHALLAT[2], O. DAVID[2,3], *B. Y. PIALLAT, PhD[4,3];
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Abstract: Mesial temporal lobe epilepsy (mTLE) consists in a paroxysmal neuronal synchronous hyperactivity within the temporal lobe, especially in the hippocampus (Hc). Because this structure is part of the Papez circuit, we hypothetize that the ictal activity would propagate through the various structures of the Papez circuit, namely the anterior nucleus (AN), what would contribute to ictal activity maintenance. To verify the involvement of AN in mTLE, we recorded the neuronal activity of AN during mTLE on epileptic monkeys. In 4 monkeys (M1 to M4), penicillin (1950-2600 UI) was injected into the Hc, on demand, at the speed of 2μL/min) using a mechanical pump connected to a Hamilton syringe, to induce the mTLE for about five hours. Temporal seizures were monitored using one Hc quadripolar macro-electrode. The AN activity was studied using a chronic implanted macroelectrode to record local field potentials in M1-3 and using a microelectrode with a recording chamber in M4. For each neuron, we calculated the mean firing rate and evaluated the type of firing pattern (rhythmic oscillatory, irregular or regular) during interictal and ictal periods. All monkeys exhibited the first ictal spike during the penicillin injection about 3 min after the beginning of the injection and the first seizure appeared during the first 30 min. The duration of seizures was of about 30s (29.3 ± 1.2 s from M2, with 3-4 seizures by 20min for 4 hours). Seizures were associated with behavioral effects, including salivation, tongue automatisms, chewing and highly frequent orofacial movements. In M1-3, we observed, within the AN, the occurrence of an ictal activity synchronized with all mTLE ictal events recorded in Hc. For all seizures, time frequency analysis showed the same frequency composition in the Hc and the AN, with, in M1, a significant oscillation at 40Hz in the AN for few seconds at the end of seizures. In M4, 8/30 AN recorded neurons showed a significant increase of firing rate time locked to the Hc interictal spikes and during the Hc seizures. We also observed that Hc interictal spikes induced, 80 to 200 ms after, significant biphasic evoked potentials within the AN region. These data show that AN neurons are indeed involved during mTLE seizures. This confirms, in non-human primate, that AN could be a good target for therapeutic neuromodulation.

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Poster

594. Epilepsy: Animal Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 594.20/J15

Topic: B.11. Epilepsy

Support: Supported by the Research Council (CDCHT) of the Univ. Centroccidental Lisandro Alvarado.

Title: Ethanol inhibits cooling-induced spinal seizures

Authors: *N. L. DALO[1], J. C. PIÑA-CRESPO[2];
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Abstract: The isolated spinal cord is capable of generating patterns of tonic-clonic seizures similar to those observed in intact animals and paroxysmal seizure-like activity could be induced by cooling the isolated spinal cord; an experimental model of seizures that depends on release of excitatory amino acids (EAA)**.** We examine whether clinically relevant doses of ethanol can prevent the onset and severity of spinal seizures. Tonic-clonic seizures of spinal origin were generated by sudden cooling to 7 ºC of the isolated spinal cord-hindleg preparation of toads. The characteristic phases of seizures and their intensity were assessed by recording muscle contractions. The onset and duration of seizures were measured 45 min after intralymphatic administration of ethanol at doses of 1.5, 2.5 and 5 g/kg diluted to 10% with Ringer solution. The tonic phase of seizures was effectively shortened or eliminated in a dose dependent manner when ethanol was given at 1.5 and 2.5 g/kg. At doses of 5 g/kg ethanol abolished all phases of seizures. The latency of seizure onset was enhanced by 71% and 145% at ethanol doses of 1.5 and 2.5 g/kg, respectively. The effect of ethanol on the pattern of seizures was compared with that of known antagonists of EAA receptors. We concluded that ethanol inhibition of the tonic phase was linked to inhibition of NMDA receptors, while inhibition of the clonic phase of seizure was due to its blocking action on AMPA receptors; on the other hand, its effect on the latency of seizure onset resembled that of drugs that enhance $GABA_A$ receptor activity.

Disclosures: N.L. Dalo: None. **J.C. Piña-Crespo:** None.

Poster

594. Epilepsy: Animal Models

Location: Halls B-H

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Program#/Poster#: 594.21/J16

Topic: B.11. Epilepsy

Title: Mitochondrial translocation of high mobility group box 1 facilitates LIM kinase 2-mediated programmed necrotic neuronal death

Authors: H.-W. HYUN, A.-R. KO, J.-E. KIM, *T.-C. KANG;
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Abstract: High mobility group box 1 (HMGB1) acts a signaling molecule regulating a wide range of inflammatory responses in extracellular space. HMGB1 also stabilizes nucleosomal structure and facilitates gene transcription. Under pathophysiological conditions, nuclear HMGB1 is immediately transported to the cytoplasm through chromosome region maintenance 1 (CRM1). Recently, we have reported that up-regulation of LIM kinase 2 (LIMK2) expression induces HMGB1 export from neuronal nuclei during status epilepticus (SE)-induced programmed neuronal necrosis in the rat hippocampus. Thus, we investigated whether HMGB1 involves LIMK2-mediated programmed neuronal necrosis, but such role is not reported. In the present study, SE was induced by pilocarpine in rats that were intracerebroventricularly infused with saline, control siRNA, LIM kinase 2 (LIMK2) siRNA or leptomycin B (LMB, a CRM1 inhibitor) prior to SE induction. Thereafter, we performed Fluoro-Jade B staining, western blots and immunohistochemical studies. LIMK2 knockdown effectively attenuated SE-induced neuronal death and HMGB1 import into mitochondria accompanied by inhibiting nuclear HMGB1 release and abnormal mitochondrial elongation. LMB alleviated SE-induced neuronal death and nuclear HMGB1 release. However, LMB did not prevent mitochondrial elongation induced by SE, but inhibited the HMGB1 import into mitochondria. The efficacy of LMB was less effective to attenuate SE-induced neuronal death than that of LIMK2 siRNA. These findings indicate that nuclear HMGB1 release and the subsequent mitochondrial import may facilitate and deteriorate programmed necrotic neuronal deaths. The present data suggest that the nuclear HMGB1 release via CRM1 may be a potential therapeutic target for the programmed necrotic neuronal death induced by SE.

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Poster

594. Epilepsy: Animal Models

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Program#/Poster#: 594.22/J17

Topic: B.11. Epilepsy

Title: Environmental enrichment reduces seizure frequency in adolescent rats prone to sound-induced seizures

Authors: *M. C. ZRULL, D. I. ALEWEL, H. C. SKINNER, S. L. SANTIAGO, H. L. JOHNSON, S. J. SNOUSE, T. J. ARNOLD;
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Abstract: Environmental enrichment (EE) promotes brain plasticity through interaction with objects and conspecifics and can protect from results of brain damage as well as mitigate severity of deficits after brain injury. Seizures, which involve abnormal neural activity that may generalize and provoke convulsions, produce brain injury with each event leading to more severe epilepsy in the future. We examined the effects of EE on audiogenic seizure (AGS) frequency and severity using a model of acquired recurrent AGS through adolescence. One group of AGS-prone Long Evans rats (n=21) lived in standard cages and received EE for 1.5-h 18 times from postnatal day (pnd) 32 to 60, and a control group of AGS-prone rats (n=18) remained in standard cages, received no EE, but were picked up periodically to control for handling. For all rats, 12 audiogenic responses (AGRs) were induced with 120 dB broadband noise on the same schedule between pnd 32 and 62. Severity of the seizure disorder was measured as latency to AGRs (wild running, shorter is more severe), and intensity of AGSs was measured as the duration of clonus (longer is more intense). When regressed on EE condition and induction session, 11% of the variance AGR latency was explained (p<.05) with latency decreasing by 52% for control and by 26% for EE rats exhibiting AGRs. Similarly, 8% of the variance in AGS duration was explained by the regression on EE and induction (p<.05) with seizure duration remaining unchanged across inductions for control and decreasing slightly (-6%) for EE rats exhibiting AGSs. While 86% of controls had seizures at the final inductions, only 50% of EE rats exhibited AGSs at these inductions (p<.05). One hour after the final induction, seizure-prone rats were sacrificed and brains were processed to visualize a neural activity marker, c-fos protein, in the lateral amygdala (LA), which is a critical relay in the network allowing anomalous excitation to reach the forebrain. EE brains revealed an increase of neural activity of 954% over baseline while control LA showed an increase of 509%. While not changing the rate of AGR occurrence or intensity of seizures when they occurred, EE did reduce the number of seizures experienced. The LA data are intriguing with EE enhancing rather than suppressing LA activity evoked by AGR induction as the behavioral data might predict; however, the activity may be of inhibitory interneurons and bares further investigation. Particularly during the critical developmental period of adolescence,

EE may provide a relatively simple, benign, non-invasive alternative to pharmacological or surgical treatment for seizure disorder, which may produce unintentional brain injury.

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Poster

594. Epilepsy: Animal Models

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Topic: B.11. Epilepsy

Support: NIH NINDS #R37NS071785-06A1

Title: Functional integration of transplanted MGE-derived interneurons within hippocampal circuits revealed using optogenetics

Authors: *J.-Y. HSIEH, S. BARABAN;
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Abstract: Interneuron-based cell therapy has gained an increasing amount of attention owing to its promising potential to rectify diseased neural networks, including epilepsy. Our previous work showed that transplanting embryonic GABA progenitors, derived from the medial ganglionic eminence (MGE), into epileptic mice suppresses spontaneous seizures and improves learning behavior (Baraban et al. PNAS 2009; Hunt et al. Nat. Neurosci. 2013). It is thought that the therapeutic effect results from synaptic integration of these cells in host circuits. However, details underlying integration of MGE-derived interneurons have yet to be fully explored. Here, by employing optogenetic and electrophysiological approaches, we further investigated the properties of grafted interneurons and the functional impact of them on local circuits in a cell-type specific manner. Embryonic MGE cells were transplanted into the hippocampus of WT mice at postnatal day 2. ChR2-eYFP was expressed in MGE-derived donors driven by GAD2-, or PV- or SST-Cre. We first characterized intrinsic properties of MGE-derived interneurons starting at 30 days after transplantation, and found that they share common attributes with their corresponding types of native interneurons. We then focused on the postsynaptic connections between MGE-derived interneurons and native pyramidal cells; spontaneous IPSC frequency and the kinetics of light-evoked IPSCs were analyzed. As expected, IPSC frequency was increased in regions containing transplanted MGE-derived interneurons. In all cases, optogenetic activation of MGE-derived ChR2-expressing interneurons evoked IPSCs on native pyramidal neurons with normal kinetics; light-evoked IPSCs were blocked by the subsequent addition of gabazine (a

GABA receptor antagonist). Next, we explored the impact of excessive activation of grafted interneurons on native pyramidal neurons. We used input-output curves to probe changes in excitability and in the dynamic range of spiking upon synchronized interneuron activation. Optogenetic activation of MGE-derived ChR2-expressing interneurons in the vicinity of a native pyramidal cell right-shifted its I-O curve. This parallel shift suggests that MGE-derived interneurons alter the circuit by simply imposing an inhibitory shunt to pyramidal cell activation and not affecting the gain (slope) or the dynamic range (max frequency). Taken together, our results show that MGE-derived interneurons functionally integrate into host circuits in a cell-type specific manner, and that even with excessive activation transplanted cells do not alter host circuit function in a non-specific, nor deleterious, manner.

Disclosures: J. Hsieh: None. **S. Baraban:** None.

Poster

594. Epilepsy: Animal Models

Location: Halls B-H

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Program#/Poster#: 594.24/K1

Topic: B.11. Epilepsy

Title: 1400W, a highly selective inducible nitric oxide synthase inhibitor is a potential disease modifier in the rat kainate model of temporal lobe epilepsy

Authors: *T. THIPPESWAMY[1], S. PUTTACHARY[2], S. SHARMA[2], S. VERMA[2], Y. YANG[2], M. R. E. PUTRA[2], A. THIPPESWAMY[2], L. DIUO[2];
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Abstract: *Status epilepticus* (SE) initiates epileptogenesis to transform normal brain to epileptic state which is characterized by spontaneous recurrent seizures (SRS). Prior to SRS, progressive changes occur in the brain soon after SE, for example, loss of blood-brain barrier (BBB) integrity, neuronal hyper-excitability (epileptiform spiking), neuroinflammation [reactive gliosis, high levels of reactive oxygen/nitrogen species (ROS/RNS)], neurodegeneration and synaptic re-organization. Our hypothesis was that modification of early epileptogenic events will alter the course of disease development and its progression. We tested the hypothesis in the rat kainate model of chronic epilepsy using a novel disease modifying drug, 1400W, a highly selective inhibitor of inducible nitric oxide synthase (iNOS). In an *in vitro* mouse brain slice model, using a multi-electrode array system, co-application of 1400W with kainate significantly suppressed kainate-induced epileptiform spiking. In the rats, *in vivo*, four hours after the induction of SE with kainate, 1400W (20 mg/kg, i.p.) was administered twice daily for three days to target early events of epileptogenesis. The rats were subjected to continuous (24/7) video-EEG monitoring,

remotely, for six months from epidurally implanted cortical electrodes. The 1400W treatment resulted in >90% reduction in SRS when compared to the vehicle-treated control group (257±113 versus 19±10 episodes) during the six month period. Immunohistochemistry (IHC) of brain sections at seven day and six month revealed a significant reduction in; reactive astrogliosis and microgliosis (M1 type), extravascular serum albumin (marker for BBB leakage) and neuro-degeneration in the hippocampus, amygdala and entorhinal cortex in the 1400W-treated rats when compared to the vehicle control. In the seven day group, hippocampal Western blots revealed downregulation of inwardly-rectifying potassium (K_{ir} 4.1) channels and glutamate transporter-1 (GLT-1) levels in the vehicle group, and 1400W treatment partially reversed K_{ir} 4.1 levels, however, GLT-1 levels were unaffected. In the six month group, a significant reduction in mossy fiber staining intensity in the inner molecular layer of the dentate gyrus was observed in the 1400W-treated group. Overall these findings demonstrate that 1400W potentially modifies epileptogenesis and the severity of the chronic epilepsy.

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Poster

594. Epilepsy: Animal Models

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Program#/Poster#: 594.25/K2

Topic: B.11. Epilepsy

Title: Chronic branched-chain amino acid ingestion aggravates hilar neuronal loss in a rodent model of temporal lobe epilepsy

Authors: S. E. GRUENBAUM[1], R. DHAHER[2], A. RAPUANO[3], A. TANG[2], *T. EID[2];
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Abstract: Introduction: The branched chain amino acids (BCAAs) valine, leucine, and isoleucine are essential amino acids that are diet-derived. There is increasing evidence to suggest that BCAAs are critical for several functions in the brain, including glutamate synthesis (and thus glutamine and GABA synthesis), intracellular signaling, immune modulation and mitochondrial health. However, there are conflicting data on the role of the BCAAs in the pathophysiology of brain disorders such as epilepsy. The objective of this study was to determine the effects of chronic BCAA ingestion on hilar neuronal loss in a rodent model of mesial temporal lobe epilepsy (MTLE). **Methods:** Sixteen rats were randomly divided into two groups: 8 rats drank a 4% aqueous solution of all three BCAAs (BCAA group) *ad libitum* for 31 days, and the other 8 rats drank regular water (control group) for the same period. After 10 days of

drinking, a microinjector was surgically implanted in the right dentate gyrus to continuously infuse the glutamine synthetase inhibitor methionine sulfoximine (MSO) for 28 days. After 31 days of drinking, rats were perfused transcardially with 0.9% NaCl followed by 4% formaldehyde in phosphate buffer. The brains were removed and fixed, sectioned on a Vibratome at 50-µm thickness, and were mounted on a gelatin-coated slides and stained with NeuN. Neuron counts in the hilar region were performed ipsilateral to the infusion site using a stereological technique. **Results:** Rats in the BCAA group had 37% fewer neurons in the dentate hilus than the control group ($5.8 \times 10^{-4} \pm 6.8 \times 10^{-5}$ vs $8.9 \times 10^{-4} \pm 5.6 \times 10^{-5}$ cells respectively, $p<0.01$). **Conclusions:** This study demonstrates that chronic ingestion of BCAAs aggravates hilar neuronal loss in a rodent model of MTLE. This study gives important insight into how BCAAs may affect neuronal viability. Although the role of BCAAs in seizure formation is unknown, these results suggest that BCAAs may play an important role in neurochemical modulation and neurotoxicity.

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Poster

594. Epilepsy: Animal Models

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Topic: B.11. Epilepsy

Support: NSERC

Title: Effect of chronic seizures on the functional integration of adult born neurons

Authors: A. KALININA, J. CARR, H. TURNER, H. LEHMANN, *N. M. FOURNIER; Dept. of Psychology, Trent Univ., Peterborough, ON, Canada

Abstract: Adult neurogenesis is a developmental process encompassing the birth of new neurons and their integration into the existing neuronal circuitry. As new neurons begin the process of integration, they pass through a transient period of heightened plasticity, which is called the critical period. This period lasts ~2 to 4 weeks in rats, and it is during this time that newborn cells are more excitable than their mature counterparts enabling these new neurons to play a key role in supporting hippocampal functions, such as spatial learning and memory. However, with aberrant stimulation, this critical period might be significantly compressed resulting in adult-born neurons integrating and becoming functionally quiescent at earlier time points. To test this possibility, we examined the impact of chronic seizures on the temporal progression of network

integration of new neurons. We labeled dividing cells with the proliferation marker BrdU before commencing with pentylenetetrazole (PTZ) kindling for a period of 1 or 2 weeks. To examine integration and activity of newborn and mature neurons, we examined for the expression of immediate early gene markers of neural activity (IEGs: Fos and Zif268) following 30-minute exposure to novel spatial environment. As expected, exploration of a novel environment significantly increased the number of Fos+ and Zif268+ cells in the dentate gyrus of non-kindled controls. In contrast, rats that received 1 or 2 weeks of PTZ kindling showed fewer Fos+ and Zif268+ cells in the dentate following exposure to the novel environment. Preliminary co-localization analysis showed that significantly fewer BrdU+ cells were activated following exploration of the novel environment in kindled rats compared to non-kindled controls. These findings raise the possibility that chronic seizures might interfere in the ability of new neurons to integrate and functionally participate in behavioural circuits. We are currently assessing the effect of longer durations of kindling on network integration of adult-born neurons as well as examining the role of GABA signaling in regulating the plasticity of new neurons in the epileptic brain.

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Poster

594. Epilepsy: Animal Models

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Topic: B.11. Epilepsy

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NIH R01 NS082046

Title: Dentate granule cell hyperactivity contributes to cognitive comorbidities in temporal lobe epilepsy

Authors: ***J. B. KAHN**[1], C. YUE[4], H. TAKANO[4,2], D. A. COULTER[1,3,4];
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Abstract: Although epilepsy management focuses on ameliorating seizure activity, patients must cope with a host of complicated cognitive comorbidities, which many patients find more detrimental to daily life than the seizures. However, the neural mechanisms mediating these

cognitive impairments in temporal lobe epilepsy (TLE) are not well defined. The dentate gyrus (DG) is fundamental for cognitive functions in the hippocampus, a structure that TLE seizures highly activate, particularly pattern separation. Pattern separation is mediated by remarkably sparse activation in the DG's granule cells (DGCs). Sparse DGC activation has a secondary effect: the DG limits cortical input to the hippocampus and acts like a "gate," the failure of which may contribute to the excessive cortical-hippocampal activity underlying TLE seizures. Our laboratory previously found a more than 10-fold increase in DGC activation patterns following epilepsy onset in our TLE mouse model. In this study, we used Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to hyperactivate DGCs in otherwise normal mice to isolate the potential cognitive consequences on a hippocampal memory task. In the spatial object recognition (SOR) task, mice are exposed to 3 objects; 24 hours later, one object is moved, and the amount of time the mice explore the displaced object (DO) reflects if the mice recognize a change in their spatial environment. Wild type mice performed well on the SOR task, spending significantly more time exploring the DO compared to the non-displaced objects (NDOs). However, epileptic mice failed to discriminate. Mice that received viral injections of the excitatory DREADD to the dorsal DG were given either saline or CNO injections i.p. 1 hour before the SOR testing trial. The saline group successfully discriminated between the DO and NDOs, while the CNO group failed to discriminate. hM3D-receiving mice were a transgenic line in which fos drives expression of tdTomato when tamoxifen is present, called Fos Targeted Recombinase in Active Populations (FosTRAP). FosTRAP is an inducible, permanent marker of activity, providing a "snapshot" of the number of DGCs that were recruited during the behavior, and confirming that CNO did not induce seizure-like activity. Voltage sensitive dye recordings in slices confirmed that CNO increases the number of DGCs that fire in response to stimulation. These data collectively suggest that DREADD-driven hyperactivation of the DGCs was sufficient to compromise behavioral performance. Thus, DGC hyperactivation may be a critical factor in TLE cognitive comorbidities.

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Poster

595. Epilepsy: Models

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Topic: B.11. Epilepsy

Support: Fapesp

Title: Transcriptome profile of the dentate gyrus and CA3 in the pilocarpine model of temporal lobe epilepsy

Authors: *A. MATOS[1], A. VIEIRA[1], A. CANTO[1], C. ROCHA[1], V. PASCOAL[2], B. CARVALHO[1], R. GLIOLI[1], I. LOPES-CENDES[1];
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Abstract: It is well known that gene expression profile of specific tissue provides relevant biological information about molecular mechanisms potentially involved in complex biological phenomena. Gene expression in different subset of cells, it is important to take sub-regional specificities, especially in the CNS. The aim of this study was to analyze and correlate gene expression profile using next generation sequencing technology in different sub-regions of the dentate gyrus (DG) and *Cornu Ammonis* 3 (CA3) in an animal model of temporal lobe epilepsy induced by pilocarpine. Male Wistar rats were injected with methyl-scopolamine (1 mg/kg) thirty minutes before of the systemic injection of pilocarpine hydrochloride (320 mg/kg) to reduce peripheral cholinergic side effects. Four hours after the administration of pilocarpine diazepam was administrated (4 mg/kg) in order to stop seizures. Control rats were injected with saline after methyl-scopolamine injection. Fifteen days after induction, rats were euthanized (n=4) and brains were processed for laser microdissection. Dorsal and ventral DG, and dorsal, intermediate and ventral CA3 were collected from each rat. RNA sequencing was performed in an Illumina Hiseq® platform. Sequences were aligned and quantified with the TopHat/DESeq2 pipeline for total RNA. Gene ontologies were analyzed with the MetaCore® software. We found a total of 969, 308, 2624, 1731 and 1278 genes differentially expressed ($p<0.05$) when comparing control and pilocarpine rats for the dDG, vDG, dCA3, iCA3 and vCA3 respectively. Gene ontology analysis indicates a predominance of inflammation related molecules among the genes found to be upregulated and synaptic transmission in genes found to be downregulated in both dDG and vDG. In addition, in the dDG there was a significant downregulation of gene in the calcium transport network, as well changes in expression of various genes involved in neuropeptides signaling, potassium and sodium transport. In dCA3 we observed upregulation of genes related to cytoskeleton remodeling and cell cycle. In iCA3 we identified upregulation of genes involved in oligodendrocyte differentiation in adult stem cells. In vCA3 there was downregulation of glutamatergic neurophysiological process, and upregulation of genes related to regulation of G1/S transition. The present data indicates region specific molecular mechanisms taking place in the hippocampus sub-regions of an animal model of temporal lobe epilepsy induced by pilocarpine. The transcriptome data suggest an interaction among several molecular components leading to epileptogenesis in this animal model that displays widespread hippocampal damage.

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Poster

595. Epilepsy: Models

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Topic: B.11. Epilepsy

Support: NC123240.1

Title: Reciprocal effect in different stages of amygdaloid kindling and forced swim test over convulsive activity and depression-like behaviour

Authors: *D. U. GONZALEZ-MENDEZ, A. DÍAZ-JIMÉNEZ, J. D. AYALA-RODRÍGUEZ, B. A. GARAY-CORTES, R. FERNÁNDEZ-MAS, A. VALDÉS-CRUZ;
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Abstract: It has been seen a high incidence of depression in patients with temporal lobe epilepsy but the biologic nature of this relationship has been poorly understood. Furthermore, there is a controversy if depression is being developed in early stages of epileptogenesis or if a depressive state during epileptogénesis allows the epilepsy easier to installate. The aim of the present study was to analyse the effect of different stages of amygdaloid kindling (according to Racine's scale) over the induced depression by forced swim test (FST) and the presence of this one over the development of epilepsy. We used male Wistar rats: 8 rats were separated in order to have a control FST group and they only did the FST. 24 rats were implanted with a tripolar electrode placed in basolateral left amygdala, these animals were divided in 4 groups: Sham group (Sham), rats implanted but with any stimulation, manipulated for 6 days and subjected to FST between manipulation days 3 and 4; control kindling (control K); kindling stage 2 + FST (K2+FST), animals subjected to FST in stage II; and kindling stage 5 + FST (K5+FST), animals subjected to FST in stage V. In last 3 groups the rats were daily stimulated until achieve the stage V for 5 days. To kindling, we analysed: number of stimuli to reach stage V, permanence, spikes, frequency spikes and duration for each stage. It was also compared the initial and final threshold to trigger spikes and seizures. To FST, we analysed the time that the animal showed immobility, swimming and climbing. In kindling, there was an increase in K2+FST in comparison to control K in number of days to achieve the first seizure ($p<0.015$), permanence of stage 3 ($p<0.021$), number of spikes in stage 4 ($p<0.036$), and spikes frequency in stage 2 ($p<0.03$), stage 3 ($p<0.002$) and stage 5 ($p<0.001$), whereas there was a reduction in seizure duration in stage 4 ($p<0.019$). In addition, there was a reduction between initial threshold and final threshold to trigger spikes ($p<0.001$) and seizures ($p<0.004$) for control K, and a reduction in final threshold to trigger spikes $p<0.037$) for K5+FST. In FST, immobility showed a reduction in K2+FST ($p<0.022$) and Sham ($p<0.008$) in comparison to control FST as well as a reduction in comparison to K5+FST (K2+FST ($p<0.008$) and Sham ($p<0.003$)). In swimming, there was a

reduction in K2+FST ($p<0.013$) in comparison to control FST. By last, in climbing was a reduction in K5+FST ($p<0.001$) in comparison to Sham. The results suggest that FST interferes with the normal establishment of kindling and susceptibility of seizures in early stages but causes more severe seizures and partial seizures and discrete damage in amygdala affects the installation of depression-like behaviour.

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Poster

595. Epilepsy: Models

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Program#/Poster#: 595.03/K7

Topic: B.11. Epilepsy

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Title: Electrical seizure threshold measurement in mu opioid receptor knockout mice

Authors: *T. N. FERRARO[1], G. A. DOYLE[3], W. H. BERRETTINI[3], R. J. BUONO[2];
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Abstract: Several lines of evidence support a relationship between opioid neurotransmission and seizure susceptibility or epilepsy. Preclinical studies document that endogenous opioid peptides and prototypical opioid drugs alike can mediate both pro- and anti-convulsant effects. Clinical reports document that seizures may be elicited as an adverse side effect of opioid agonists including morphine and meperidine, as well as the opioid antagonists naloxone and naltrexone. Moreover, genetic association studies provide suggestive evidence that variants in *OPRM1*, the gene that encodes the mu opioid receptor, influence the risk for developing epilepsy in humans. To further elucidate the role of endogenous opioids in regulating the expression of seizure activity, we measured electrical seizure threshold in strains *Oprm1* knockout (MuKO) mice in which the null allele was transferred to both C57BL/6J (B6) and DBA/2J (D2) genetic backgrounds. Allelic transfer was accomplished with a backcross breeding strategy involving use of the neomycin cassette insert sequence, together with an *Oprm1* sequence spanning the gene promoter and exon 1, as selection markers. Heterozygous MuKO mice were brother –sister mated after 10 backcross generations to produce homozygous null *Oprm1* mice for this study. MEST was measured in 8-10 week old mice by administering a single daily electrical stimulus (via earclip electrodes) with a constant current electroshock unit (Ugo Basile, model 7801). The

starting current was 20 mA (MuKO-D2) or 40 mA (MuKO-B6) and the current level was increased 1 mA per day. Seizures were scored as focal, generalized or maximal (i.e. tonic extension of hind limbs). Whereas the stimulus current was increased with each daily trial, stimulus frequency (60 Hz), pulse width (0.4 ms), and duration (0.2 s) remained constant. Testing revealed that MuKO-B6 mice have a significantly lower MEST compared to parental B6 mice, with similar results obtained for females and males. On the other hand, there is no significant difference in MEST between MuKO-D2 and parental D2 mice, possibly due to a floor effect mediated by the D2 genetic background. Consistent with prior studies, B6 females were found to have a lower seizure threshold than B6 males; this difference is not observed in D2 mice. Overall, we conclude that the mu opioid receptor is involved in regulating seizure threshold in certain strains of mice, and that molecules involved in opioid neurotransmission represent potentially viable targets for developing new epilepsy pharmacotherapies.

Disclosures: **T.N. Ferraro:** None. **G.A. Doyle:** None. **W.H. Berrettini:** None. **R.J. Buono:** None.

Poster

595. Epilepsy: Models

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NIH/NINDS K08NS069667

Tross Epilepsy Research Fund University of Iowa

Title: Neurochemical and behavioral effects of chronic stress in a mouse model of temporal lobe epilepsy

Authors: *J. B. DE ROSS[1,2], N. K. LEIBOLD[2], V. K. MENDOZA[2], G. F. BUCHANAN[2]; [1]Univ. of Sao Paulo, Ribeirao Preto, Brazil; [2]Neurol., Univ. of Iowa, Iowa city, IA

Abstract: Depression is one of the most frequent comorbidities in patients with temporal lobe epilepsy (TLE). It is suggested that these conditions have a bidirectional relationship, however, the mechanisms underlying this association are poorly understood. It is known that chronic stress worsens epileptogenesis and seizure outcome, although the possible vulnerability of the epileptic brain to chronic stress has not been properly addressed. Deficits in serotonin (5-HT) and dopamine (DA) neurotransmission may be involved in both conditions and can predispose

individuals to both seizures and depressive symptoms. Thus, with this work we aimed to investigate some of the behavioral and neurochemical aspects of the relationship between depression and epilepsy and how the epileptic brain responds to chronic stress. Adult male C57BL/6J mice with 6 weeks of age were subjected to the pilocarpine-status epilepticus model of TLE (PILO) or saline treatment (CTRL), and were implanted with EEG electrodes and microdialysis cannulae directed toward the hippocampus. Once the pilocarpine-treated animals developed spontaneous recurrent seizures, half of the animals in each treatment group were subjected to an unpredictable chronic mild stress (UCMS) paradigm and the other half of each group underwent sham housing. All animals were then evaluated for stress hormone levels, exploratory activity, depressive-like behavior, $5HT_{1A}$ receptor function and changes in 5-HT and DA release after intrahippocampal citalopram administration. Prior to the UCMS paradigm, PILO animals presented with higher corticosterone levels and hyperlocomotion, compared to CTRL. After UCMS, PILO animals spent less time at the center of the open field, showed an enhanced immobility time in the tail suspension test, reduced food intake, a blunted hypothermic response to 8-OH-DPAT injection and a reduction in 5-HT and DA release in the hippocampus after local citalopram perfusion. These data indicate that chronic seizures seem to enhance stress hormones levels in mice, which in turn, show a more prominent depressive-like phenotype and reduced monoamine release in the hippocampus after chronic stress. In conclusion, this study corroborated our hypothesis that the epileptic brain is more vulnerable to detrimental effects of stress, mechanism that may underlie the high prevalence of depression among TLE patients. Further examination into the underlying mechanisms of these changes is underway.

Disclosures: **J.B. De Ross:** None. **N.K. Leibold:** None. **V.K. Mendoza:** None. **G.F. Buchanan:** None.

Poster

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Support: NINDS (No. 5R01NS094550)

NINDS (No. 5R21NS084182)

NIGMS (2P20GM103432)

Title: Continuous spike-waves during slow-wave sleep (CSWS) in a mouse model of focal cortical dysplasia (FCD)

Authors: *C. ZHOU, Q.-Q. SUN, W. YANG, C. ZHANG, D. PETRUS;
Zoology and Physiol., Univ. of Wyoming, Laramie, WY

Abstract: Objective To examine if mice with focal cortical dysplasia (FCD) develop chronic spontaneous epileptic seizures and if so, what are the key EEG features in comparison to that reported in similar clinical conditions. **Methods** Unilateral single freeze lesions to the S1 region (SFLS1R) were made in postnatal day 0–1 pups to induce a neocortical microgyrus in the right cortical hemisphere. Continuous 24 hour recordings with screw-free intracranial EEG electrodes and a battery of behavioral tests were performed in adult SFLS1R and sham control mice to assess neurological status. **Results** 88% (30/34) of SFLS1R and 0% (0/11) of control, age matched mice developed chronic spontaneous ictal-spikes (IS). 20 of the 30 SFFLS1R mice with IS activities exhibited highly frequent, persistent and repeatable spontaneous electrographic seizures that occurred predominantly during the NREM sleep. The epileptic discharge pattern closely resembled the pattern of continuous spike-waves during slow-wave sleep (CSWS) of the human epileptic syndrome described as an electrical status epilepticus during slow wave sleep (ESES). Electrographic seizures were strongest in the right S1 region though generalized to contralateral cortex, hippocampus and thalamus, and were associated with significant cognitive and behavioral deficits. Seizures were temporarily alleviated by ethosuximide treatment or optogenetic activation of cortical S1 GABAergic neurons. **Interpretation** This is the first report of a FCD model displaying chronic CSWS/ESES type EEG seizures. Further characterization of the abnormal corticocortical and corticothalamic oscillations in this model may lead to a better understanding of the mechanisms and treatment of CSWS/ESES in humans.

Disclosures: C. Zhou: None. **Q. Sun:** None. **W. Yang:** None. **C. Zhang:** None. **D. Petrus:** None.

Poster

595. Epilepsy: Models

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Topic: B.11. Epilepsy

Support: NIH Grant

Title: Cortical hyperexcitability and loss of local field potential synchrony in a mouse model of focal cortical dysplasia

Authors: *A. J. WILLIAMS, W. YANG, C. ZHOU, Q.-Q. SUN;
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Abstract: Focal cortical dysplasias (FCDs) are a common cause of brain seizures and are often associated with intractable epilepsy. Here we evaluated aberrant brain neurophysiology in an *in vivo* mouse model of FCD induced by neonatal freeze lesions (FLs) to the right cortical hemisphere (near S1). Linear multi-electrode arrays were used to record extracellular potentials from cortical and subcortical brain regions near the FL in anesthetized mice (6-9 months old) followed by 24h cortical EEG recordings in awake animals. Results indicated that FL animals exhibit a high prevalence of spontaneous spike-wave seizure discharges, predominately during sleep (EEG), and a reduced threshold for burst-suppression activity under general anesthesia (extracellular recordings, 0.5-3.0% isoflurane). Brief periods of burst activity in the local field potential (LFP) included an increase in theta-alpha spectral peaks (4-12 Hz) on a background of low-amplitude delta activity (1-4 Hz). Burst activity was more prominent in the superficial vs. deep cortical layers, was associated with an increase in spontaneous spiking of cortical neurons, and was highly synchronized in control animals across both cortical and subcortical layers (average cross-correlation values ranging from +0.73 to +1.0) with minimal phase shift between electrodes and strong signal coherence below 20 Hz. However, in FL animals, cortical vs. subcortical LFP signals were strongly out of phase with significantly lower cross-correlation values compared to controls (average values of -0.1 to +0.5, $P<0.05$ between groups). In particular, a marked reduction in the level of synchronous LFP activity was observed the closer the recording electrodes were to the FL (Pearson's Correlation = 0.525, $P<0.05$). In summary, these studies indicate the presence of altered extracellular signal patterns near the site of cortical FLs that may disrupt the synchronous flow of information between cortical and subcortical brain regions. Cortical FLs were also associated with an increased pattern of hyperexcitable burst activity that may represent an altered level of excitation to inhibition in the FL brain contributing to the associated spike-wave seizure discharges observed in these animals.

Disclosures: **A.J. Williams:** None. **W. Yang:** None. **C. Zhou:** None. **Q. Sun:** None.

Poster

595. Epilepsy: Models

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Support: Medical Research Council L01095x

Title: Comparison of tetanus toxin rat models for gene therapy for focal neocortical epilepsy

Authors: *E. CHABROL, A. SNOWBALL, A. LIEB, R. C. WYKES, S. SCHORGE, M. C. WALKER, D. M. KULLMANN;
UCL, London, United Kingdom

Abstract: Focal neocortical epilepsy is frequently resistant to medication, and surgical resection is rarely feasible, often because of proximity to eloquent cortex. New treatment options are therefore needed.
We reported successful lentiviral-mediated gene therapy in a rat model of focal motor epilepsy (Wykes et al., 2012). Tetanus toxin (TeNT) injected into the motor cortex elicited frequent, brief (<1 s), spontaneous bursts of high frequency epileptiform EEG activity, which lasted over 1 month, and at higher doses were accompanied by focal and occasionally secondarily generalized motor convulsions. Seizures were suppressed by lentiviral overexpression of the potassium channel Kv1.1, which causes a reduction in neuronal excitability and neurotransmitter release. Although gene therapy was effective against focal motor epilepsy, it is not known if this can be generalized to epilepsy arising in other brain areas, which is important for clinical translation. We implanted rats with wireless EEG transmitters (Open Source Instruments, Inc), which permit continuous monitoring. TeNT injected into the rat visual cortex was followed within 3-4 days by the occurrence of spontaneous seizures lasting 30-80 seconds and occurring approximately 10 times a day for as long EEG recordings were performed (>1 month). Importantly, these seizures were well tolerated with minimal morbidity. Simultaneous video recordings showed frequent generalization. TeNT in visual cortex leads to longer and less frequent seizures than TeNT in motor cortex. Although the motor cortex TeNT model resembles epilepsia partialis continua, an especially refractory form of epilepsy, the seizures in the visual cortex model are more typical of secondarily generalized seizures.

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Poster

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CURE

Epilepsy Foundation

Tross Epilepsy Research Fund University of Iowa

Title: Differential seizure susceptibility and postictal cardiorespiratory outcomes depending on vigilance state and genetic ablation of serotonin neurons

Authors: *B. S. PURNELL[1,2], K. CLAYCOMB[3], S. KRUSE[1], G. F. BUCHANAN[1,2]; [1]Neurol., [2]Interdisciplinary Grad. Program in Neurosci., Univ. of Iowa, Iowa City, IA; [3]Neurol., Yale Sch. of Med., New Haven, CT

Abstract: Amongst neurological conditions SUDEP, or sudden unexpected death in epilepsy, is second only to stroke in terms of years of potential life lost. Due to the unforeseen nature of SUDEP it is difficult to determine its antecedents; however, we know that SUDEP usually occurs at night and follows a generalized tonic-clonic seizure. What makes the seizures which precede SUDEP different from those that epilepsy patients suffer with more regularly is poorly understood. Using maximal electroshock seizures (MES) we have previously demonstrated that vigilance state can alter parameters such as seizure likelihood, severity, respiration and survivability. We have also shown using MES that modifications to the serotonin system can alter seizure outcomes. MES experiments are limited by the fact that MES is an acute model of seizures in a seizure-naïve brain which has not undergone the changes in excitability which characterize epilepsy. In this investigation we assessed whether the changes to seizure susceptibility and postictal cardiorespiratory outcomes which were seen in the MES model would extend to two models of epilepsy: amygdala kindling and pilocarpine-temporal lobe epilepsy. In the amygdala kindling group wildtype (WT) mice and mice lacking central serotonin neurons (*Lmx1b*$^{f/f/p}$) were implanted with EEG, EMG and EKG electrodes as well as a bipolar electrode in the right basolateral amygdala. Mice were kindled with twice daily stimulations (80-240 mA, 1 ms biphasic square wave, 1 s, 60 Hz). Once fully kindled, seizures were elicited during rapid eye movement sleep (REM), non-rapid eye movement sleep (NREM) and wakefulness. Seizures and cardiorespiratory sequelae were characterized using EEG, EMG, EKG, and plethysmography recordings. In the temporal lobe epilepsy group status epilepticus was induced using pilocarpine resulting in subsequent spontaneous seizures. These animals were monitored via plethysmograph, EKG, EEG and EKG to determine the vigilance state and cardiorespiratory outcomes of the spontaneous seizures. In both WT and *Lmx1b*$^{f/f/p}$ mice seizures induced by amygdala kindling during NREM resulted in an increase in respiratory rate variability and an increased occurrence of apneas. Seizures were rarely inducible during REM in WT mice but were more readily induced during REM in *Lmx1b*$^{f/f/p}$ mice. Spontaneously occurring REM seizures were rare in the WT mice but were more frequent in the *Lmx1b*$^{f/f/p}$ mice. This data suggests that seizures occurring during sleep can have adverse effects on respiration which may result in death. Also, this investigation implicates serotonin in the vigilance state dependent nature of seizure susceptibility and severity.

Disclosures: **B.S. Purnell:** None. **K. Claycomb:** None. **S. Kruse:** None. **G.F. Buchanan:** None.

Poster

595. Epilepsy: Models

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Support: Italian Ministry for Research, FAR

Italian Ministry for Research, PRIN

Title: Increased frequency of up-states in neocortical slices of synapsin triple knock-out epileptic mice at presymptomatic ages

Authors: *L. FORTI[1], A. LOCARNO[2], G. POLITA[4], G. BRESCHI[5], V. GNATKOVSKY[7], E. MONZANI[6], F. C. GUARNIERI[6], F. BENFENATI[8], L. MUZIO[5], R. FESCE[3], F. VALTORTA[9]; [1]Dept. of Biotech. and Life Sci., [2]Neurobio. PhD Course, [3]Dept.Theoretical and Applied Sci., Univ. of Insubria, Busto Arsizio, Italy; [4]Master in Biomed. Physics, Univ. of Pavia, Pavia, Italy; [5]Neuroimmunology Unit, INSpe, Div. of Neurosci., [6]Div. of Neurosci., San Raffaele Scientific Inst., Milano, Italy; [7]Fondazione Inst. Neurologico C.Besta, Milano, Italy; [8]Italian Inst. of Technol. and Univ. of Genova, Genova, Italy; [9]San Raffaele Scientific Inst. and Vita Salute Univ., Milano, Italy

Abstract: Synapsins (Syns) are a family of synaptic vescicle-associated phosphoproteins involved in synaptic development, function and plasticity (Cesca et al., 2010). Mice with deletion of the Syn I,II and III genes (triple knock-out, TKO) are a validated model of epilepsy (Boido et al., 2010; Ketzef et al., 2011), with spontaneous and evoked seizures which start to occur around two months of age (Cambiaghi et al., 2013). The lack of synapsins in these mice was shown to generate specific alterations at excitatory and inhibitory synapses (Ketzef et al., 2011; Farisello et al., 2013) and hippocampal hyperexcitability (Boido et al., 2010) at both the pre-symptomatic and symptomatic ages.

We investigated the presence of alterations of neocortical network excitability at presymptomatic ages. We focused on the synchronous, low frequency (<1 Hz) propagating network activity recorded in cortical slices with extracellular electrodes, shown to correspond to the up- and down-states detected in individual pyramidal neurons (Sanchez-Vives & McCormick, 2000). This activity has been proposed to be the slice counterpart of the slow oscillations observed with intracellular and EEG recordings during sleep, anesthesia, and some phases of quiet wakefulness. We studied the occurrence of up-states in somatosensory cortex slices from age-matched C57BL/6J wild type (*wt*) and TKO mice (P24-P36) using microelectrode array recordings of extracellular voltage. Up-states had significantly higher frequency in TKO mice. We also measured their layer of origin, duration, amplitude and power spectral density, as well as their apparent spatial extension and propagation velocity. Up-states appeared to originate with larger

probability in supragranular layers in *wt*, but with more homogeneous probability across layers in TKO; similarly, the total power of activity was generally maximal in supragranular layers in *wt* mice, while in TKO mice in a substantial fraction of cases maximum activity was observed in granular or infragranular layers. Up-state duration and spatial extent were similar in *wt* and TKO mice. Overall, this study shows the presence of altered neocortical network excitability in TKO mice at presymptomatic ages.

Disclosures: **L. Forti:** None. **A. Locarno:** None. **G. Polita:** None. **G. Breschi:** None. **V. Gnatkovsky:** None. **E. Monzani:** None. **F.C. Guarnieri:** None. **F. Benfenati:** None. **L. Muzio:** None. **R. Fesce:** None. **F. Valtorta:** None.

Poster

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Support: NIH Interagency Agreement with USAMRICD

Title: Age-dependent susceptibility to seizure and neuronal loss in rat soman model

Authors: F. ROSSETTI[1], M. F. STONE[2], M. C. MOFFETT[2], T. M. TURNER[2], L. S. NAIDU[2], *B. MARRERO-ROSADO[2], C. R. SCHULTZ[2], L. A. LUMLEY[2];
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Abstract: Introduction: *Status epilepticus* (SE) occurs at double the rate in those over 65 years of age. Although epilepsy increases with age, few studies have evaluated the development of epilepsy in aged rats. We recently observed that aged rats exposed to the chemical warfare nerve agent soman (GD) are more sensitive to the lethal effects of GD compared to adult rats. Aged rats have loss of hippocampal interneurons, and reduced inhibition may increase excitability, which may lead to increased susceptibility to development of seizures. We currently report on the effects of GD exposure in adult and aged male rats on seizure activity and neuronal loss. Methods: Male F344 adult (2 months old) and aged (18 months old) rats were implanted with telemetry devices for monitoring EEG, temperature and activity. Rats were exposed subcutaneously (sc) to equitoxic doses (88 and 66 μg/kg, respectively) of soman (GD) and treated 1 min later with standard medical countermeasures (atropine sulfate and oxime HI-6) and 30 min after seizure onset with the anticonvulsant diazepam. Rats were monitored continuously for the development of SE and early recurrent seizures (eRS) in the first 3 days, as well as the

development of spontaneous recurrent seizures (SRS) until 60 days after exposure, and performance was assessed in a radial arm maze. Results: Although aged rats received a lower dose of GD compared to adult rats, aged rats tended to have a shorter onset to SE, spend more time in SE+eRS, have an earlier onset of SRS, and have a greater number of SRS. Both young adult and aged rats exposed to GD that developed seizures had an equally significant reduction in density of glutamic acid decarboxylase 67 (GAD-67), a marker of GABAergic interneurons, in the basolateral amygdala and piriform cortex. Aged rats tended to have less GAD-67 cells than young adult rats, mainly in the hippocampus and amygdala. Both young adult and aged rats tended to have reduced GAD-67+ cells in the dentate gyrus of the hippocampus. However, the reduction was higher in aged rats compared to young adult rats. Conclusion: Clearly, the sensitivity to GD exposure is age-dependent. Aged rats are highly susceptible to the effects of soman on SE, SRS or the development of epilepsy and consequent brain pathology. *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. This research was supported by an interagency agreement between the USAMRICD and NIH.

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Poster

595. Epilepsy: Models

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Topic: B.11. Epilepsy

Support: CURE SUDEP Grant 2014

Title: Cardiorespiratory dysfunctions and sudden death in two mouse models of intractable epilepsy

Authors: A. M. BARD, N. SAHAI, S. M. HANNA, J. SKIBO, A. ROY, J. RAMIREZ, K. J. MILLEN, *F. K. KALUME;
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Abstract: Sudden unexpected death in epilepsy (SUDEP) is the most common type of death in people with intractable epilepsies. These include epilepsy associated with focal cortical dysplasia (FCD) and Dravet syndrome (DS). FCD is a developmental disorder with early childhood onset marked by regional brain malformations and intractable seizures. FCD type IIa has been linked

with gain-of-function mutations in *PIK3CA, a* gene involved in development and cancer. Conversely, DS is a treatment-resistant epilepsy with infantile-onset and elevated SUDEP rates. DS is often caused by loss-of-function mutation in *SCN1A*, the gene encoding Na_V 1.1 channels. SUDEP is caused by cardiovascular dysfunctions. We conducted dual examinations of cardiac and respiratory functions during interictal, ictal, and post-ictal periods to identify and compare signs of SUDEP susceptibility in a mouse model of FCD (carrying a gain-of-function mutation in Pik3ca) and that of DS (harboring a heterozygous knock-out of Scn1a). Video-EEG-ECG and whole body plethysmograph were recorded from these mutant mice and controls using fine silver EEG, ECG and EMG electrodes. Signals were acquired on a Power Lab 8/35 using LabChart Software 8.0 (AD Instruments). In DS mice, our previous studies showed a substantial suppression of resting heart rate variability, increased frequency of AV blocks, and no change in resting heart rate (HR). In these studies, we observed suppressed respiratory responses to hypercapnia (60 ± 8 %), hypoxia (48 ± 5 %), and anoxia (31 ± 6 %) in DS mice (n=6) compared to WT (n=9). In addition, these mice exhibited concurrent transient bradycardia and bradypnea during the tonic phases of thermal generalized tonic clonic seizures. Mice carrying DS-causing mutation in GABAergic interneurons alone, not excitatory neurons, revealed similar ictal and interictal dysregulations of cardiorespiratory functions. In FCD mice (n=6), resting interictal recordings also showed a decreased in HRV, but accompanied with increase in HR and absence of AV blocks. Respiratory recordings showed reduced respiratory responses to hypercapnic (80 ± 9 %), hypoxic (60 ± 8 %), and anoxic (30 ± 7 %) conditions compared to controls (n=6). Furthermore, PTZ-induced seizures caused less severe physiological abnormalities than to those of DS mice. These results suggest that regardless of the etiology of epilepsy, seizures cause similar cardiorespiratory dysfunctions leading to SUDEP. GABAergic interneurons are implicated in the mechanisms of SUDEP. Understanding the cellular and network mechanisms underlying these physiological dysfunctions may lead to better therapeutic methods for SUDEP prevention.

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Poster

595. Epilepsy: Models

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Support: 309011

Title: Effect of growth hormone on status epilepticus induced by lithium-pilocarpine in adult rats

Authors: *I. ZAMORA-BELLO[1], E. VELAZCO CERCAS[1], A. PUIG LAGUNES[1], L. BELTRAN PARRAZAL[1], C. MORGADO VALLE[1], I. SANTIAGO ROQUE, 91010[2], E. JUAREZ AGUILAR, 91010[3], M. LOPEZ MERAZ[1];
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Abstract: It has been reported that growth hormone (GH) facilitates epileptogenesis induced by electrical kindling in mice. However, some kinases implicated in GH signaling pathways, such as ERK and PI3K, are associated with pro- and anti-convulsant effects. The goal of this study was to evaluate the effect of intracerebroventricular administration of GH on *status epilepticus* (SE). Adult Wistar male rats were implanted with a guide cannula into the right ventricle and GH was injected at different amounts (70, 120 y 220 ng) for 5 days using artificial cerebrospinal fluid as a vehicle. SE was induced by the lithium-pilocarpine model (3 mEq/kg and 30 mg/kg, respectively) 24 h after the last administration of GH. Rats were injected with diazepam (10 mg/kg) one hour after the SE onset. Seizure severity was evaluated with the Racine´s scale. Data were analyzed by a proportions test or one-Way ANOVA followed by a Tukey post-hoc test. All rats (100%) injected with 120 ng of GH required a greater number of injections of pilocarpine (2.7 ± 0.8) to develop SE compared with the other GH groups ([0%] 1 ± 0.8 for 70 ng GH and [20%] 1.2 ± 0.7 for 220 ng GH) and the vehicle group ([16.6%] 1.2 ± 0.4). The latency to the first generalized seizure stage IV/V (79.3 ± 7.2 min) and SE (84.1 ± 6.8 min) was higher in the group of 120 ng GH when compared with the 70 ng GH (49.6 ± 10.2 min and 55.6 ± 9.7 min, repectively), 220 ng GH (43.2 ± 20.4 min and 47.4 ± 19.2 min, respectively) and the vehicle (42.9 ± 13.7 min and 49.2 ± 12.2 min, respectively) groups. No differences in the duration of SE or generalized seizures as well as in the number of generalized seizures stage IV and V were observed between the experimental groups. In conclusion, the subchronic administration of GH showed an anticonvulsant effect against lithium-pilocarpine-induced SE.

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Poster

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Topic: B.11. Epilepsy

Title: The role of hippocampal oxygen levels in the development and expression of epilepsy in two models: intrahippocampal kainate and perforant path electrical stimulation.

Authors: *M. D. WOLFF, M. SCANTLEBURY, G. C. TESKEY;
Univ. of Calgary, Calgary, AB, Canada

Abstract: *Rationale:* We recently determined in the kindling model of focal seizures, that following the cessation of brief electrographic seizures, a long-lasting, severe hypoxic event occurs in the brain regions involved in the electrographic seizure. For example, local tissue oxygenation drops below 10 mmHg and remains at a severe hypoxic level for over an hour in the hippocampus. We reasoned that given this severe and long-lasting change in oxygen levels following a brief ictal event, there might also be changes in local oxygen levels during the induction of epilepsy; these changes in turn may account for the development of chronic epilepsy in rodent models. Current animal models of epilepsy employ the use of either electrical stimulation or a chemoconvulsant in order to induce spontaneous recurrent seizures (SRS) in rodents. Our aim was to conduct a head-to-head comparison of both models with the goal to prevent status epilepticus, limit lethality, and ultimately produce self-generating seizures in a rodent. We hypothesized that during the induction of epilepsy, there will be drastic changes in hippocampal oxygen levels. *Methods:* A sub-anesthetic dose of urethane was administered before the induction protocols in order to sequester electrical activity to the temporal lobes and prevent generalized status epilepticus. In the intrahippocampal kainic acid model, kainic acid was infused directly into the rat ventral hippocampus. In the electrical stimulation model, a 24-hour stimulation protocol of the perforant path was used (Norwood et al. 2010). In both groups, oxygen levels and EEG were recorded in dorsal hippocampus throughout the first 24 hours. Immediately after the 24-hour induction period, rats were transferred to a 24/7 video-EEG monitoring unit. Hippocampal EEG was monitored continuously for 4-6 weeks. Once per week oxygen recordings were taken alongside EEG to look for spontaneous seizure-induced hypoxia. *Analysis:* Several dependent measures were recorded and analyzed. Firstly, we analyzed EEG to look at the frequency, duration, and severity of seizures starting from the first day post-induction. We also analyzed oxygen levels post-induction in order to determine if frequent episodes of post-ictal hypoxia were related to the development of SRS. *Significance:* This study will advance our current understanding of epilepsy models. In addition, we hope to uncover the role that oxygen may play towards the development of SRS in these models. These discoveries may lead to the development of new treatments or preventative strategies for people with epilepsy.

Disclosures: **M.D. Wolff:** None. **M. Scantlebury:** None. **G.C. Teskey:** None.

Poster

595. Epilepsy: Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 595.14/L1

Topic: C.09. Brain Injury and Trauma

Support: NINDS Grant NS065877

NINDS Grant NS033310

Title: Gamma event coupling as a measure of functional connectivity during epileptogenesis

Authors: *L. LI[1], J. ALMAJANO[1], J. ENGEL, Jr[1,2,3], A. BRAGIN[1,2];
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Abstract: **Rationale:** Epilepsy is one of the most serious neurological diseases and it is becoming increasingly apparent that epileptic activity may cause alterations of local and distant functional connections within neuronal networks. However, whether, and what types, of connectivity reorganization can lead to epilepsy after potentially epileptogenic brain lesions, is unknown. This study aims to investigate if coupling of local field potentials (LFP) in the gamma band range (30-55Hz) changes after kainic acid (KA) lesions of hippocampus and neocortex and, if so, whether these changes correlate with the process of epileptogenesis. **Methods:** Experiments were conducted on adult male Sprague-Dawley rats (n = 21). 16 tungsten microelectrodes (50 um outer diameter) and screw electrodes were implanted 1 week in symmetrical sites of prefrontal cortex, motor cortex, hippocampus and thalamus**.** KA was injected either in the left anterior hippocampus or left neocortex. Two-month continuous EEG recordings were performed with sampling frequencies from 1k to 10k Hz. Maxima for gamma activity waves were detected and Shannon entropy was applied to determine the functional connectivity between different brain regions as peri-event histograms for each pair of channels. Connectivity index (**CI**) represented by normalized Shannon entropy values between areas of interest, was then compared to distinguish the connectivity differences between rats that developed seizures within two months after brain lesioning, and rats that did not. **Results:** An overall decrease in regional connectivity was observed in both groups after two-months of recordings, compared to the baseline. We observed a decreased **CI** in rats without seizures, between the peri-lesion site and the left prefrontal cortex as well as the right thalamus, and an increased **CI** between the peri-lesion site and right prefrontal cortex, right hippocampus and left thalamus**,** forming a balanced gamma event coupling strength between the left and right sides of the brain. Rats with seizures showed a decreased **CI** between the lesion site and most of the ipsilateral regions, and an increase **CI** between the lesion site and contralateral regions, revealing a shift of gamma event coupling strength to the opposite site of the lesion. **Conclusion:** Our

results indicate **CI** changes after local epileptogenic lesions of specific brain areas. Spatial reorganization of functional connectivity of the perilesional area with local and remote networks was different in animals that did, and did not develop epilepsy. Patterns of gamma event coupling changes could be biomarkers for prediction of epilepsy in patients after potentially epileptogenic brain injury.

Disclosures: **L. Li:** None. **J. Almajano:** None. **J. Engel:** None. **A. Bragin:** None.

Poster

595. Epilepsy: Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 595.15/L2

Topic: B.11. Epilepsy

Support: NIH Grant R01 DK056132-12

Title: Sudep and functional remodeling of vagal complex activity in a mouse model of temporal lobe epilepsy

Authors: *I. DERERA[1], B. P. DELISLE[1,2], B. N. SMITH[1,2];
[1]Univ. of Kentucky, Lexington, KY; [2]Univ. of Kentucky, Epilepsy Ctr., Lexington, KY

Abstract: Sudden unexpected death in epilepsy (SUDEP) is the most common cause of death in individuals with refractory epilepsy and has been associated with disturbances in cardiorespiratory function and autonomic nervous system (ANS) imbalance. GABAergic neurons of the nucleus tractus solitarius (NTS) of the brainstem dorsal vagal complex are essential modulators of parasympathetic tone. Studies of genetic models of epilepsy suggest that ANS dysfunction coincides with SUDEP susceptibility, but this has not been investigated in models of temporal lobe epilepsy (TLE), the most common epilepsy type. This study investigated cardiac function and activity of GABAergic NTS neurons in the vagal complex in the pilocarpine-induced status epilepticus (pilo-SE) model of TLE. Pilocarpine (281 mg/kg) was administered to 5-6 week old mice to induce SE and eventual development of TLE. GIN mice were split into 3 groups to assess survival rates, in vivo electrocardiography (ECG), and *in vitro* electrophysiology of GABAergic NTS neurons at several times post-SE. Pilocarpine-treated mice displayed a 30% survival rate (versus 100% for vehicle-treated controls) by 150 days post-SE. Heart rate was increased and heart rate variability was decreased by 12 weeks post-SE relative to age-matched control mice. For electrophysiological recordings from identified GABAergic NTS neurons, coronal brainstem slices were taken at 1, 6, and 12 weeks post-SE. Significant increases in spontaneous action potential and EPSC frequency ($p<0.05$) were

detected 1, 6, and 12 weeks post-SE. Miniature EPSC frequency was increased relative to controls at 12 weeks post-SE, but not at 1 and 6 weeks. Blockade of ionotropic glutamate receptors significantly reduced spontaneous action potential frequency to levels similar to controls at 6 and 12 weeks post-SE. These results suggest long-term changes in cardiac function develop in conjunction with increased synaptic excitability of GABAergic NTS neurons after pilo-SE, which could contribute to autonomic dysregulation, cardiorespiratory collapse, and SUDEP. Future studies will determine if increased excitability of GABAergic NTS neurons underlies increased susceptibility to depolarization block and spreading depression in the NTS of mice with TLE.

Disclosures: I. Derera: None. **B.P. Delisle:** None. **B.N. Smith:** None.

Poster

595. Epilepsy: Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 595.16/L3

Topic: B.11. Epilepsy

Support: Citizens United for Research in Epilepsy

Title: Malfunction of beta-catenin pathways leads to infantile spasms and seizures

Authors: *A. PIRONE, J. ALEXANDER, L. ANDRESEN, C. DULLA, M. JACOB;
Dept. of Neurosci., Tufts Med. Sch., Boston, MA

Abstract: Infantile Spasms (IS) is a catastrophic childhood epilepsy syndrome, characterized by neonatal flexion-extension motor spasms, which progress to chronic seizures and cognitive deficits in later life. The underlying molecular and functional changes that cause IS are poorly defined and animal models remain sparse. Genetic screens of individuals with IS have identified multiple risk genes, several of which are predicted to alter β-catenin pathways. β-catenin has dual roles in the N-cadherin synaptic adhesion complex and the canonical Wnt signaling pathway- both are critical for the normal formation and function of brain circuits. Tests for a link between malfunction of β-catenin pathways and IS are lacking. Here, we show that mice with conditional deletion in neurons of adenomatous polyposis coli protein (APC cKO), the major negative regulator of β-catenin levels, leads to excessive β-catenin and most of the features of human IS. Compared with wild-type littermates, neonatal APC cKOs exhibit flexion-extension motor spasms and abnormal electroencephalographic (EEG) activity. Cortical hyperexcitability is also indicated by increased spontaneous and evoked excitatory electrical activity in layer 5 pyramidal neurons at postnatal day 9, the age of peak spasm intensity. At adult ages, APC cKOs

display spontaneous behavioral seizures and abnormal EEGs. Further, we show that our novel mouse model with conditional overexpression of β-catenin that lacks the degradation domain (β-cat cOE) also leads to excessive β-catenin levels and IS-like phenotypes (neonatal spasms and chronic seizures). Both APC cKO and β-cat cOE mice show increases in synaptic spine density, abnormal plasticity of excitatory synapses, and changes in circuit connectivity. In particular, cortico-striatal connections are altered in our two different genetic mouse models of IS. Our data provide the first *in vivo* evidence for a novel molecular etiology of IS that is centered on aberrant β-catenin networks. Defining new molecular targets is essential for developing novel and effective therapeutic interventions to ameliorate IS and seizures.

Disclosures: **A. Pirone:** None. **J. Alexander:** None. **L. Andresen:** None. **C. Dulla:** None. **M. Jacob:** None.

Poster

595. Epilepsy: Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 595.17/L4

Topic: B.11. Epilepsy

Title: Seizure-induced brainstem hypoxia's involvement in sudden unexpected death in epilepsy

Authors: ***A. K. WALL**[1,4], J. S. FARRELL[2,4], G. C. TESKEY[3,4];
[2]Neurosci., [3]Cell Biol. and Anat., [1]Univ. of Calgary, Calgary, AB, Canada; [4]Hotchkiss Brain Inst., Calgary, AB, Canada

Abstract: Sudden unexpected death in epilepsy (SUDEP), which occurs when an individual dies with no identifiable cause of death, is the leading cause of death directly related to epilepsy. Recent clinical research suggests that SUDEP results from a failure to breathe during or following a seizure, but the mechanism behind this failure is unknown. We have recently discovered a period of local tissue hypoxia (< 10mmHg), that occurs during and following a seizure in the brain areas involved in the seizure which is severe enough to cause behavioural dysfunction. This seizure-induced hypoxia provides a potential mechanism for the breathing failure that leads to death in cases of SUDEP; seizure activity invades brainstem breathing centers leading to local hypoxia, cellular/synaptic dysfunction of breathing centers, breathing failure, and death.
We hypothesized that seizure activity would propagate from the hippocampus to brainstem breathing centers, leading to severe hypoxia in this region preceding breathing failure and death. To investigate we administered intra-hippocampal kainic acid to adult male mice to induce seizures and death. Following kainic acid administration we continuously recorded seizure

activity, brainstem oxygen levels, breathing, and heart rate in awake, freely moving mice. Seizure activity in brainstem breathing centers was measured from chronically implanted bipolar electrodes. Tissue oxygen levels in brainstem breathing centers were measured during seizures and death using chronically implanted fiber-optic oxygen probes. Breathing was measured using a nasally-implanted thermocouple, and heart rate using a bipolar electrode implanted across the heart. This research provides preclinical data regarding the relationship between seizure activity, brainstem oxygen levels, breathing, heart rate, and death underlying SUDEP.

Disclosures: **A.K. Wall:** None. **J.S. Farrell:** None. **G.C. Teskey:** None.

Poster

595. Epilepsy: Models

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Program#/Poster#: 595.18/L5

Topic: B.11. Epilepsy

Support: 2011/50680-2; 2014/11277-6, Sao Paulo Research Foundation –FAPESP

Title: Brain proton magnetic resonance spectroscopy and T2-weighted in the pilocarpine model of temporal lobe epilepsy: changes after status epilepticus in rats

Authors: *R. BARBOSA[1], A. S. VIEIRA[2], A. H. B. DE MATOS[2], B. M. DE CAMPOS[1], R. F. CASSEB[1], L. RAMALHO PIMENTEL-SILVA[1], B. CARVALHO[3], R. GILIOLI[4], I. LOPES-CENDES[2], F. CENDES[1];
[1]Dept. of Neurol., [2]Dept. of Med. Genet., [3]Inst. of Mathematics, Statistics and Computing Sci., [4]Multidisciplinary Ctr. for Biol. Investigation on Lab. Animal Sci., Univ. of Campinas, Campinas, Brazil

Abstract: Our aim was to evaluate *in vivo* changes in T2-weighted MRIs and proton magnetic resonance spectroscopy (^{1}H-MRS) in the pilocarpine (PILO) model of hippocampal sclerosis. We studied 31 Wistar rats with ^{1}H-MRS and T2-weighted images acquired in a 3-T Philips scanner with an 8 integrated channels volumetric coil (Rapid Biomedical GmbH, Wurzburg, Germany). Spectra were obtained in the hippocampus using a single voxel with point-resolved spectroscopy (PRESS) at TE/TR: 135/2000ms. We acquired MRI prior to any intervention and then 48 hours, 15 and 30 days after pilocarpine induced status epilepticus (SE). After the first MRI, we induced seizures with intraperitoneal injections of PILO hydrochloride (320mg/kg). Thirty minutes prior to pilocarpine the animals received scopolamine (1mg/kg) to reduce systemic cholinergic side effects. After four hours of the onset of SE, we administrated diazepam (4mg/kg) to interrupt seizures. The sham-treated were injected with saline after scopolamine

injection. The metabolite quantification was analyzed by LCModel software. Only good quality spectra (with <15% of error using Cramér-Rao lower bounds) were included. Metabolites were expressed in terms of their ratio to Creatine+Phosphocreatine. Statistical analysis was performed using SPSS software. PILO group had significant lower values of the following compounds when compared to the sham-treated group: in MRI2 (cholines [GPC+PCh], t=-4.48, p<0.001; N-acetylaspartate [NAA+NAAG], t=-8.17, p<0.001; glutamate+glutamine [Glu+Gln], t=-6.58, p<0.001), MRI3 (GPC+PCh, t=-6.14, p<0.001; NAA+NAAG, t=-7.64, p<0.001; Glu+Gln, t=-7.44, p<0.001) and MRI4 (GPC+PCh, t=-5.97, p<0.001; NAA+NAAG, t=-7.94, p<0.001; Glu+Gln, t=-8.16 p<0.001). The visual assessment of repeated MRIs showed a progressive increase of T2 hippocampal signal. We showed progressive decrease of the neuronal marker NAA, as well as cholines and Glu+Gln, in addition to increased T2 signal intensity in the hippocampi 48 hours, 15 and 30 days after pilocarpine induced SE. Further correlations of these changes with EEG and histology will help to understand better the epileptogenic damage in this model of hippocampal sclerosis.

Disclosures: **R. Barbosa:** None. **A. S. Vieira:** None. **A. H. B. de Matos:** None. **B. M. de Campos:** None. **R. F. Casseb:** None. **L. Ramalho Pimentel-Silva:** None. **B. Carvalho:** None. **R. Gilioli:** None. **I. Lopes-Cendes:** None. **F. Cendes:** None.

Poster

595. Epilepsy: Models

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Program#/Poster#: 595.19/L6

Topic: B.11. Epilepsy

Support: NSF CAREER AWARD 1149446

Title: Functional role of neuronal hyaluronic acid in the naked mole-rat

Authors: *D. THEVALINGAM[1,2], D. MCCLOSKEY[1,2];
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Abstract: Molecules that compose the extra cellular matrix regulate synaptic plasticity and may mediate the ionic microenviroment at the neuron membrane. Notably, Hyaluronic Acid (HA) is involved in a number of cellular processes that modulate neuronal excitability as well as CNS development. Previous work has shown that tissues in the African Naked Mole-Rat, including brain, accumulate an exceptionally high amount of HA, likely due to decreased activity of digestion enzymes, which confers early contact inhibition and cancer resistance in this species

[Tian et al. (2013) Nature. 499(7458): 346-349]. The purpose of the present study was to explore the functional outcome of HA accumulation in the naked mole-rat brain. Anatomical measures of the extracellular matrix (through Wisteria floribunda agglutinin staining) and HA (through immunohistochemical labeling of Hyaluronic Acid Binding Protein) demonstrate robust labeling of multiple cell types throughout the naked mole-rat brain, including hippocampus. In the naked mole-rat hippocampal slice, which demonstrates epileptiform hyperexcitability under routine recording conditions, application of the HA digestion enzyme, hyaluronidase, increased the frequency of epileptiform burst discharges. Thus, as in other species, HA digestion increases epileptiform activity in the naked mole-rat. The role of HA in buffering the naked mole-rat intracellular pH, an important factor in naked mole rat neuron excitability, and the sensitivity to HA digestion across the long lifespan of the naked mole-rat, are currently under investigation.

Disclosures: D. Thevalingam: None. **D. McCloskey:** None.

Poster

596. Brain Wellness and Aging: Models and Mechanisms

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 596.01/L7

Topic: C.01. Brain Wellness and Aging

Support: R01AG043467

Title: Aged females exhibit blunted c-Fos induction in response to social interaction.

Authors: *T. DEAK[1], A. E. PERKINS[1], A. C. TOMCZIK[2], E. R. WOODRUFF[2], L. E. CHUN[2], R. L. SPENCER[2];
[1]Behavioral Neurosci. Program, Dept. of Psychology, Binghamton University-SUNY, Binghamton, NY; [2]Dept. of Psychology and Neurosci., Univ. of Colorado Boulder, Boulder, CO

Abstract: Aging is accompanied by a decline in the expression of social behavior, with females showing a more pronounced aging-related decline than males. Although the mechanisms underlying these changes are not clear, prior studies have noted a general hyporeactivity to stimuli among aged rats. Thus, in the present study, we used *c-Fos* induction as a marker of neuronal activation to identify key sites relevant to the processing of sensory stimuli (generally) or social stimuli (specifically) after brief exposure to a social partner. To do this, 3- and 18-month-old female F344 rats (N = 60; n = 10/group) were left undisturbed in their home cage as controls (HCC), exposed to a testing context alone for 30 min (CXT), or were exposed to the context for 20 min, followed by a 10 min social interaction (SI) test with a young conspecific. CXT and SI animals were pre-exposed to the context prior to test day to allow habituation to a

novel environment. This design allowed us to separate neuronal activation to a testing environment (CXT) from activation specific to social stimuli (SI). Tissue was collected 30 min after the end of testing to assess *c-Fos* induction with *in situ* hybridization. Both the context and social interaction led to a significant increase in *c-Fos* expression in the barrel field cortex (BF), hippocampus (HPC), and medial amygdala (MeA), brain regions that play a role in sensory processing and social behavior regulation. In contrast to our previous findings in aged males, aged females exhibited blunted *c-Fos* induction in response to a social interaction compared to 3-month-old females in BF, HPC, and MeA. These data indicate that although young females exhibit substantial neuronal activation specifically in response to social stimuli, the same is not true for aged females. In fact, aged females exhibit similar levels of *c-Fos* induction, regardless of testing condition, although this response is blunted compared to young females. This pattern was observed across all structures examined, supporting the idea that aged animals exhibit hyporesponsivity to stimuli, although this appears to be sexually dimorphic. Future studies will focus on females to examine whether age-related neuroinflammation may suppress neuronal activation in these brain regions.

Disclosures: **T. Deak:** None. **A.E. Perkins:** None. **A.C. Tomczik:** None. **E.R. Woodruff:** None. **L.E. Chun:** None. **R.L. Spencer:** None.

Poster

596. Brain Wellness and Aging: Models and Mechanisms

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 596.02/L8

Topic: C.01. Brain Wellness and Aging

Support: R01AG043467

Title: Stereological assessment of microglia number and morphology reveals sex differences in aged male and female F344 rats in socially relevant brain structures.

Authors: *A. E. PERKINS[1,2], T. A. MCNAMARA[2], T. DEAK[2];
[2]Dept. of Psychology, [1]Binghamton Univ., Binghamton, NY

Abstract: Aging is associated with a substantial decline in social behavior regulation, as well as increased neuroinflammation indicated by enhanced expression of cytokines and chemokines and an exaggerated response to peripheral immune challenges. Furthermore, there are sex differences in microglia number across the lifespan, with males exhibiting greater number of microglia during the neonatal period, whereas females show greater numbers of microglia in young adulthood. However, most studies examining microglia number or morphology in aging have

focused on the hippocampus or cortical structures, in part due to their role in age-related cognitive decline. Thus, we examined age and sex differences in microglia within brain regions critical to social behavior regulation (PVN, BNST, and MeA). Adult (3-month) and aged (18-month) male and female F344 (N = 40, n = 4-8/group) rats were perfused directly from their home cage and a subset of 18-month-old animals were injected with lipopolysaccharide (250 μg/mL) and perfused 24 h later to assess both the number and activational state of microglia as a positive control. Iba-1 immunopositive microglia were counted stereologically and classified according to the following criteria: (i) thin ramified processes, (ii) thick long processes, (iii) stout processes, or (iv) round/ameboid shape. Females, regardless of age or LPS treatment, had fewer microglia in the MeA, a brain structure that plays a significant role in social behavior regulation. Although we did not observe any differences in microglia number as a result of aging in the MeA, there appeared to be an increase in the number of round/ameboid microglia in the MeA of aged animals, suggesting increased inflammation in late aging. Thus, we hypothesize that aging may result in enhanced cytokine release, perhaps by affecting the release of socially-relevant neuropeptides. Future studies will focus on understanding the relationship between neuroinflammation and social behavior regulation, particularly within social behavior circuits.

Disclosures: **A.E. Perkins:** None. **T.A. McNamara:** None. **T. Deak:** None.

Poster

596. Brain Wellness and Aging: Models and Mechanisms

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Topic: C.01. Brain Wellness and Aging

Support: RO1AG043467

Title: The impact of housing conditions in aged Fischer 344 rats on social behavior and cytokine expression within socially-relevant circuits

Authors: ***J. E. MONDELLO**, A. E. PERKINS, E. I. VARLINSKAYA, T. DEAK;
Dept. of Psychology, Binghamton Univ., Binghamton, NY

Abstract: Housing conditions play a role in the expression of social behavior. Single-housing is often considered a form of social deprivation, whereas long-term housing with several cage mates constitutes a form of social enrichment. However, nearly all studies in rodents have tested the influence of housing conditions in early development, leaving a gap in our knowledge of how housing conditions impact social processes in late aging. Here, we assessed the influence of housing on social behavior and inflammation within brain structures implicated in the

modulation of social behavior in male and female 18-month-old Fischer 344 rats (N = 54; n = 8-10/group). We varied the number of housing partners to include 3 conditions: single, pair, and triple-housing with sex-matched 3-month-old animals. Conditions were maintained for 5-6 weeks, after which social behavior toward a novel 4-month-old conspecific was assessed. Animals were re-housed for 7 days before tissue collection under ambient conditions. Aged males pair- or triple-housed with young animals exhibited social avoidance and reduced frequency of social behavior, whereas housing condition did not impact social behavior in females. Cytokine gene expression was examined in three socially relevant brain regions: PVN, BNST, and MeA. IL-1β and IL-6 expression were significantly increased in the MeA of triple-housed females, indicative of heightened inflammatory signaling. No such increase was observed in the PVN or BNST, arguing against widespread inflammation that would suppress social behavior, as is the case with sickness. Although males exhibited significant changes in social behavior as a result of housing condition, there were no changes in cytokine expression. We hypothesize that increased cytokine activity within the MeA may alter socially-relevant neuropeptide systems, as prior research demonstrated that IL-1β administration can enhance release of oxytocin and vasopressin. This, in turn, may attenuate the suppression of social behavior that occurs as a result of housing with younger cage mates. Future studies will examine the interaction between neuropeptides and inflammation in order to better understand the mechanisms driving social behavior during senescence.

Disclosures: **J.E. Mondello:** None. **A.E. Perkins:** None. **E.I. Varlinskaya:** None. **T. Deak:** None.

Poster

596. Brain Wellness and Aging: Models and Mechanisms

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Topic: C.01. Brain Wellness and Aging

Support: P01AG026572

R01AG032236

U01AG024904

W81XWH1220012

Title: Towards multivariate genetic models predicting Alzheimer's disease risk

Authors: *B. C. RIEDEL[1], R. D. BRINTON[3,2], P. M. THOMPSON[2];
[1]Neurosci., [2]USC, Los Angeles, CA; [3]3Center for Innovation in Brain Sci. and Dept. of Pharmacology, Sch. of Med., Univ. of Arizona, Tucson, AZ

Abstract: Alzheimer's disease (AD) is a complex and multifactorial disease that ultimately leads to severe memory impairment and neuronal death. The clinical phenotype of AD is highly variable, which is indicative of multiple etiologies and progression trajectories. Consequently, up to 40% of patients living with AD in the U.S. are undiagnosed until the late stages of the disease. Identifying individuals early in the disease process is critical for reducing the substantial health burden that AD causes at the individual, familial, and societal levels. Until recently, apolipoprotein E (APOE) was the only well-established high penetrance susceptibility gene for AD. Although the heritability of AD based on twin studies is as high as 80%, APOE accounts for less than 1/3 of this estimate. Furthermore, APOE-*ε4* is neither necessary nor sufficient to cause AD, and most genetic factors influencing Alzheimer's risk are unknown. Genome-wide association studies (GWAS) have identified over 30 different AD susceptibility genes. Using the curated GWAS Catalog and the ENDEAVOR gene prioritization tool trained on the top 10 consistently replicated Alzheimer's risk genes, we identified 37 Alzheimer's risk genes that show evidence of systems biology interactions in mediating risk for the disease. Our preliminary analyses with participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) (N=1,477, mean age: 74 ± 7; 634 women, 843 men) followed for an average of 3.4 years found 9,752 variants among these 37 genes, with a minor allele frequency of .05 or greater. Individually, no risk variant was a better predictor of CSF Aβ-142, CSF phosphorylated tau levels, right or left hippocampal volume, or longitudinal clinical diagnosis of Alzheimer's, than positive carrier status of the APOE-ε4 allele. Notably, of the APOE deterministic variants, rs429358 was a better predictor of each disease-related variable than rs7412. Ongoing analyses are creating multivariate genetic models combining these risk variants in order to understand the systems biology of the disease and improve overall genetic detection and understanding of Alzheimer's.

Disclosures: B.C. Riedel: None. **R.D. Brinton:** None. **P.M. Thompson:** None.

Poster

596. Brain Wellness and Aging: Models and Mechanisms

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Program#/Poster#: 596.05/L11

Topic: C.01. Brain Wellness and Aging

Support: NIA 5P01AG026572

Title: Mechanistic role of brain hypometabolism and mitochondrial uncoupling in perimenopausal hot flash

Authors: *R. D. BRINTON[1,2], F. YIN[1], J. YAO[1], A. MISHRA[1], Q. DENG[1], Z. MAO[1], E. CADENAS[1];
[1]Sch. of Pharm., USC, Los Angeles, CA; [2]Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: The goal of the current study is to determine the mechanism of the signature symptom of the menopausal transition, the perimenopausal hot flash. We hypothesized that loss of ovarian hormone regulation of bioenergetics in brain induces a series of adaptive responses in the brain, which are initiated by decline in glucose metabolism, followed by activation of alternative fuel sources, ketone bodies and free fatty acids, which lead to mitochondrial uncoupling and temperature dysregulation. We used the ovariectomy (OVX) rat model as a reliable and predictive inducer of temperature dysregulation. Loss of ovarian hormones in the OVX rats led to decreased uterine weight, increased body weight and a significant increase in peripheral (tail skin) temperature. Further, our analyses in the OVX rat model indicated that peripheral temperature dysregulation coincided with systemic glucose intolerance and decreased cerebral glucose metabolism (FDG-PET). We further tested our hypothesis that loss of ovarian hormones leads to disruption and uncoupling of the proton motive force-dependent energy conservation systems and the consequent dissipation of energy as heat. Results of these analyses indicated that mitochondrial respiratory control ratio (RCR) was decreased in OVX rats accompanied by increased mitochondrial uncoupling, the upregulation of mitochondrial uncoupling proteins (UCPs), and enhancement of mitochondrial fragmentation in multiple brain regions. OVX-induced changes were completely or partially prevented by 17beta-estradiol treatment, suggesting an obligatory role of estrogen signaling in these events. Finally, to investigate the relationship between mitochondrial uncoupling in the brain and the increase in peripheral temperature, mitochondrial uncoupling was induced by 2,4-dinitrophenol (2-DNP), a mitochondrial uncoupler. Our analyses indicate that intracerebroventricular injection of 2-DNP induced sequential fluctuations in brain temperature, core temperature and tail skin temperature. Collectively, we established the physiological and bioenergetic phenotype of a rat model of hot flash, and our findings provide new mechanistic details of hot flash by connecting loss of ovarian hormones, brain hypometabolism, mitochondrial uncoupling and dysfunction, and peripheral temperature dysregulation. This work was supported by NIA 5P01AG026572 to RDB; Project 5 to RDB.

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Poster

596. Brain Wellness and Aging: Models and Mechanisms

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Topic: C.01. Brain Wellness and Aging

Support: NIA P01AG026572 to RDB; Project 1 to RDB & EC, Animal Core B to RDB, Analytic Core C to EC, and Administrative Core A to RDB & WM

Title: Transcriptional and epigenomic changes across the perimenopause transition

Authors: *E. BACON[1], M. K. DESAI[1], A. MISHRA[1], Y. WANG[1], F. YIN[1], R. D. BRINTON[2]; [1]USC, Los Angeles, CA; [2]Univ. of Arizon, Tucson, AZ

Abstract: Transition states represent critical periods during development when systems under go dramatic and widespread changes. In women, the perimenopause transition spans several years and the resulting loss of estrogen has profound effects in nearly all tissues, including breast, bone, cardiovascular, and brain. Menopause in humans is also marked by an increased risk for stroke, coronary heart disease, and neurological disorders. Although a majority of women have no serious long-term health consequences, many women suffer neurological symptoms during and after the perimenopause transition. Heritability of menopause timing is 44-66% and variability is present in monozygotic twins and inbred rat strains, suggesting that epigenetics and environmental factors could play a large role in orchestrating timing of events involved in reproductive aging. Individual differences in epigenetic regulation, in addition to individual differences of sex hormone levels may help explain some of the differences seen in menopausal age, risk for cognitive impairment, and response to hormone therapy. To better understand the potential underlying mechanisms of neurological symptoms associated with perimenopause, as well as the control of age of onset, the current study aims to characterize the transcriptional and epigenomic changes that occur during this transition using a rat model recapitulating fundamental characteristics of the human perimenopause. Duration of the perimenopause transition in Sprague Dawley rats (time spent cycling irregularly before loss of cyclicity) can be separated into three groups: short, average, long. All animals begin to cycle irregularly around the same time (9-10mo), however animals complete the perimenopause transition at different ages. Older ages are correlated with longer overall durations of transition. Transcriptional changes of genes related to epigenetic regulation were observed across all perimenopause groups (RC, IR, AC). Our analysis suggests that hypothalamic aging and changes in epigenetic regulation begin before the onset of irregular cycling, between 6 and 9 months. Changes in DNA methylation across the perimenopause transition were investigated via global 5mC ELISA and genome-wide bisulfite sequencing (RRBS). Furthermore, IPA upstream analysis of transcriptional data identifies key players of one-carbon metabolism (involved in epigenome

maintenance) as likely regulators of endocrine aging. Impaired one-carbon metabolism during perimenopause may contribute to increased risk for neurodegenerative diseases after menopause.

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Poster

596. Brain Wellness and Aging: Models and Mechanisms

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Topic: C.01. Brain Wellness and Aging

Support: NIA 5P01AG026572

Provost Fellowship, USC

Title: Evidence for immune cell infiltration into the brain during perimenopausal transition: Implications for autoimmunity and neurodegenerative diseases.

Authors: *M. DESAI[1], Y. CHEN[2], F. YIN[3], Z. MAO[3], R. BRINTON[3,4];
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Abstract: The onset of reproductive senescence occurs at the perimenopause, which is characterized by major physiological changes in the endocrinological and reproductive systems which can be associated with altered energy metabolism, cognition, bone-mineral density, cardiovascular function and immune system responses. Our rat model of the perimenopause to menopause to post menopause transitions captures both chronological and endocrinological conversions. In the current study, we investigated the differential regulation of genes in the hippocampi of the endocrine characterized female rats by sequencing hippocampal total RNA. We conducted paired end sequencing of hippocampal total RNA from 36 Sprague Dawley female rat hippocampi with read length of 50 base pairs and a read depth of ~50 million reads per sample using Illumina HiSeq 2500. Raw data files in the FASTQ format underwent QA/QC and trimming procedure in the cloud-based Partek Flow environment (http://www.partek.com/). The paired end reads for each sample were then aligned using TopHat to the rat reference genome rn6 (Ensembl 80). Transcript assembly and quantification of aligned reads were carried out using Cufflinks. The Cufflinks output consisted of a list of differentially expressed genes (DEG) for each comparison. Differentially expressed genes were analyzed using Ingenuity

Pathway Analysis to identify gene pathways altered during the perimenopause. Results of this curated literature based bioinformatic analysis indicated activation of pathways related to lymphocytic proliferation and differentiation during the transition from regular to irregular cycling and inactivation of pathways responsible for T cell apoptosis. RNAseq also detected a decrease in platelet-derived growth factor (PDGF) activity, associated with decreased brain estrogen, indicating potential for increased blood brain barrier permeability during the perimenopausal hippocampus. Analyses of significantly differentially expressed genes (DEG) using PANTHER (http://pantherdb.org/index.jsp) revealed enrichment of B and T lymphocyte genes. Interestingly, Interleukin-27, a pan-T lymphocyte regulator was down-regulated during the perimenopausal transition, reinforcing the hypothesis that T lymphocyte dysregulation is associated with the perimenopausal transition. These RNAseq and bioinformatic analyses extend our findings to the immune system as a potential key regulator of the perimenopause transition state. Ongoing analyses are pursuing functional evidence for increased blood brain barrier permeability, increased T cell infiltration into brain and immune dysregulation in periphery and brain.

Disclosures: **M. Desai:** None. **Y. Chen:** None. **F. Yin:** None. **Z. Mao:** None. **R. Brinton:** None.

Poster

596. Brain Wellness and Aging: Models and Mechanisms

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Program#/Poster#: 596.08/L14

Topic: C.01. Brain Wellness and Aging

Support: NIA 5P01AG026572

Title: Effects of APOE genotype on obesity-induced acceleration of Alzheimer-related pathology in female EFAD mice

Authors: ***A. CHRISTENSEN**[1], E. BACON[2], F. YIN[2], R. BRINTON[2,3], C. PIKE[1]; [1]Dept. of Gerontology, [2]Sch. of Pharm., USC, Los Angeles, CA; [3]Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: Alzheimer's disease (AD) risk is significantly influenced by genetic and environmental risk factors. The greatest genetic risk factor for late onset AD is APOE genotype, with E4 carriers being at a greater risk than E2 or E3 carriers. This increased risk in APOE4 carriers is exacerbated in females. Further, obesity has been shown to play an important role in both male and female AD risk. The potential gene-environment interactions between APOE and

obesity in regulation of AD pathogenesis are not well understood. To investigate this issue, we used the EFAD mouse model, which has humanized APOE3 (E3FAD) or APOE4 (E4FAD) in the presence of 5xFAD genes. Female transgenic mice were maintained on either Western diet (WD; 45% fat, 21% sugar) or a control diet (10% fat, 7% sugar) for 12 weeks. E3FAD mice on WD were impaired on cognitive tasks including spontaneous alternation behavior and novel object recognition. E4FAD mice were more impaired on these tasks in comparison to E3FAD mice, but showed no further impairment by WD. Plaque load in the hippocampus showed a similar pattern with WD significantly increasing beta-amyloid deposition in E3FAD females. Amyloid load was relatively higher in E4FAD versus E3FAD mice, but was not significantly increased by WD. To begin identifying factors that underlie the observed interactions between APOE and diet-induced obesity, we performed hippocampal gene microarrays in transgenic APOE3 and APOE4 mice that lack AD transgenes but were maintained on the same diet interventions. The microarray data indicate numerous changes in expression of inflammation- and metabolism-related genes across diet and APOE groups that are consistent with roles in regulation of pathology. Overall, these findings demonstrate significant gene-environment interactions between APOE and obesity in female EFAD mice. Continued investigation of how AD is affected by the interactive effects of risk factors such as obesity and APOE is essential to the identification of at-risk populations and the development of preventive strategies. (EFAD transgenic mice were generously provided by Mary Jo LaDu at University of Illinois at Chicago.)

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Poster

596. Brain Wellness and Aging: Models and Mechanisms

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Program#/Poster#: 596.09/M1

Topic: C.01. Brain Wellness and Aging

Support: NIA 5P01AG026572

Title: Ovariectomy and hormone treatment modulates female brain bioenergetic function in an endocrine aging dependent manner

Authors: ***Z. MAO**[1], F. YIN[1], J. YAO[1], E. CADENAS[1], R. BRINTON[1,2];
[1]Sch. of Pharm., USC, Los Angeles, CA; [2]Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: The perimenopause is an aging transition unique to the female that is associated with multiple neurological symptoms. Our recent study in a rodent model of human perimenopause revealed the perimenopausal transition from regular- to irregular cycling as a critical period for brain metabolic function, characterized by a significant decline in bioenergetic and synaptic functions. Combinations of estrogens and progestogens in varying regimens are widely used as hormone therapy for menopause-related climacteric symptoms. We previously reported that a two-month treatment of continuous 17β-estradiol (E2) in combination with cyclic progesterone (P4) (E2+CyP4) on ovariectomized (OVX) young rats induced a bioenergetic gene-expression profile comparable to the ovary intact females. The present study was aimed to determine the efficacy and optimal intervention window of the E2+CyP4 therapy on female rat brain at different stages of the perimenopausal transition. Placebo or E2+CyP4 therapy was initiated on female rats at 9-10 months old with either regular cycling or irregular cycling, and for each cycling status, Sham OVX or OVX surgery was performed before the intervention. Hormone therapy consisted of two 30-day cycles of continuous E2 and cyclic P4 (10 days/cycle) delivered by silastic capsules. Upon completion of the regime, brain derived samples were analyzed for genomic, biochemical and brain metabolic responses. Our data indicate that the efficacy of E2+CyP4 therapy on brain bioenergetic functions in terms of glucose metabolism and mitochondrial respiratory capacity was differentially affected by the endocrine aging state of the rats when the intervention was initiated. Interestingly, our data also suggested that OVX initiated in regular or irregular cyclers elicited opposite effects on bioenergetic- and inflammatory gene expression. Outcomes of this study in the model of natural perimenopause will determine the window of opportunity for preventing the at-AD-risk bioenergetic phenotype by hormone intervention and will provide mechanistic details for developing novel strategies to maintain neurological health and function throughout female brain chronological and endocrine aging.
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Poster

596. Brain Wellness and Aging: Models and Mechanisms

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Topic: C.01. Brain Wellness and Aging

Support: NIA 5P01AG026572

Title: Impact of APOE genotype on the bioenergetic system in perimenopausal mouse brains

Authors: *F. YIN[1], Y. WANG[1], A. MISHRA[1], Z. MAO[1], E. CADENAS[1], R. D. BRINTON[2,1]; [1]Sch. of Pharm., USC, Los Angeles, CA; [2]Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: Our previous studies in a rodent model of human perimenopause demonstrated that the perimenopause is a neurological bioenergetic transition state that is reminiscent of early stage of Alzheimer's disease (AD) and suggested that bioenergetic shifts that occur in the female aging brain contributes to an increased risk for AD in women. As one of the greatest genetic risk factors for AD, APOE4 genotype manifests stronger impact of pathology and risk of AD in females. The aim of the current study is to provide a mechanistic rationale for the adverse impact of the APOE4 gene on the female bioenergetic system shifted during normal perimenopausal transition. Preliminary gene expression analyses of humanized ApoE4 and ApoE3 targeted replacement (TR) female mice revealed that ApoE4-TR mice at pre-menopause age (6-month old) exhibited a significantly different bioenergetic gene expression profile in the hippocampus relative to the age-matched ApoE3-TR controls. Surprisingly, pre-perimenopausal ApoE4 mice exhibited a significant increase in the RNA expression of genes encoding subunits of mitochondrial complexes for oxidative phosphorylation, multiple mitochondrial inner- and outer-membrane transporters, as well as a gene that controls mitochondrial fusion, Opa1. In parallel, the ATP citrate lyase gene was downregulated, which could be interpreted as a decrease of citrate-transported acetyl-CoA units for fatty acid and cholesterol biosynthesis. It remains to be determined whether increased expression of bioenergetic genes in pre-menopause ApoE4 mice is an indicator of hypermetabolism, or it represents an adaptive response to deficits in other components of the metabolic system such as substrate uptake and utilization, or it is due to a shift in brain energy fuels from glucose to ketone bodies and fatty acids. The last hypothesis is supported by peripheral data we obtained, where ApoE4 mice have higher levels of plasma ketone bodies and triglycerides compared to the age-matched ApoE3 female controls and to the ApoE4 male mice. Outcomes of our ongoing systems biology analyses of the ApoE-perimenopause phenotypes will determine how the bioenergetic profile observed in the pre-menopause stage changes upon peri- and post-menopause, which will provide mechanistic details of the ApoE4 genetic burden on the bioenergetic fluctuation of the perimenopausal transition and its contribution to AD risks in women. This work was supported by NIA 5P01AG026572 to RDB; Project 1 to RDB & EC.

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Poster

596. Brain Wellness and Aging: Models and Mechanisms

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Topic: C.01. Brain Wellness and Aging

Support: NIA 5P01AG026572

Title: Transitions in the inflammatory phenotype during the perimenopausal transition: Implications in Alzheimer's disease

Authors: *A. MISHRA[1], M. K. DESAI[1], E. BACON[2], Y. WANG[1], F. YIN[3], E. CADENAS[3], R. D. BRINTON[4,3];
[1]Clin. therapeutics, [2]Neurosci. Grad. Program, [3]Dept. of Pharmacol. and Pharmaceut. Sci., USC, Los Angeles, CA; [4]Ctr. for Innovation in Brain Sci. and Dept. of Pharmacol., Univ. of Arizona, Tucson, AZ, CA

Abstract: Alzheimer's disease (AD) is characterized by a long latent prodromal stage, and the perimenopausal transition in women is considered a "tipping point" in the development of the AD phenotype. Characterization of the inflammatory phenotype during the prodromal phase is crucial for development of inflammation based therapeutics and biomarkers. The disparate results from the ADAPT trial showed the beneficial effects of NSAIDs treatment at an early stage but the worsening of pathology and symptoms in older adults, highlighting the changing course and function of inflammation with the disease. Both age and menopause are causative factors that affect inflammation. This study is an effort towards studying the effects of the perimenopasual transition on the emerging inflammatory phenotype and understanding its possible implications on the development of Alzheimer's disease.
In our preliminary study using the perimenopausal rat model, which isolates endocrine aging from chronological aging, we observed an increase in inflammation during pre-menopausal chronological aging in the hippocampus. We observed a significant increase in expression of MHC-II (Major histocompatibility complex-II) molecules, involved in antigen presentation: HLA-DQA1, HLA-DQB1, HLA-DRA, HLA-E, HLA-G, HLA-DRB5 during pre-menopausal chronological aging, identified by RNA-Seq analysis. Upregulation of the complement pathway is also observed during this phase. During endocrine aging, the female rats transition from regular cycling to irregular cycling followed by acyclicity, we observed a decline in the expression of MHC-II molecules, HLA-DQA1 and HLA-DQB1. In post-menopausal chronological aging, the MHC-II signaling molecules: HLA-DQA1, HLA-DQB1, HLA-DRA increased significantly. The differences in the expression of MHC-II in the hippocampus, during the course of chronological aging and the perimenopausal transition, implies that there are changes in the reactivity of key immune players: microglia, perivascular macrophages and

astrocytes. Peripherally, serum cytokine levels of IL-1α and IL-12, measured by multiplex assay, were significantly increased in acyclic 9 month old rats and acyclic 16 month old female rats, in comparison to regular cycling 9 month old rats. These data are indicative of dynamic and distinct immune signaling during both endocrine and chronological aging. Further, the distinctive inflammatory phenotype in the perimenopausal transition and chronological aging provide insights key to the development of therapeutics and biomarkers targeting risk of AD.
This work was supported by NIA 5P01AG026572 to RDB; Project 1 to RDB & EC.

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Poster

596. Brain Wellness and Aging: Models and Mechanisms

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Topic: C.01. Brain Wellness and Aging

Support: NIA Grant 5P01AG026572

Title: Mitochondrial and nuclear gene expression required for mitochondrial respiration are differentially affected by endocrine and chronological aging.

Authors: ***Y. WANG**[1], M. DESAI[1], R. BRINTON[2];
[1]Clin. and Exptl. Therapeutics, Sch. of Pharm., USC, Los Angeles, CA; [2]Ctr. for Innovation in Brain Sci. and Dept. of Pharmacol., Univ. of Arizona, Tucson, AZ

Abstract: Human mitochondrial oxidative phosphorylation system comprises five electron transport chain complexes. The system is made up of roughly 130 subunits, of which 13 core subunits are encoded by the mitochondrial genome. Alterations in gene expression can lead to changes in cellular respiration and bioenergetics, which are implicated in multiple neurodegenerative diseases including Alzheimer's disease. To study the initiation of the prodromal phase of AD, we investigated the endocrine perimenopausal transition and chronological aging. Our analyses focused on mitochondrial respiration and electron transport chain gene expression in female brain using a rat model recapitulating fundamental characteristics of the human perimenopause and aging. Specifically, female Sprague-Dawley rats between 9-10 months old were classified as either regular cycling, irregular cycling, or acyclic based on their estrus status. 6-month-old regular cycling and 16-month-old acyclic rats were included to distinguish the effects of chronological aging from endocrine aging. We used rtPCR to determine gene expression profile of mitochondrial-encoded genes and RNAseq for that of

nuclear encoded genes. Results of these analyses indicated that in the hippocampus, during perimenopause, MT-ND3 , MT-CYB , and MT-ATP6 had significantly lower expression in both irregular and acyclic 9-month-old animals compared to regular cyclic 9-month animals. MT-CO1, MT-CO2, and MT-CO3 had significantly lower expression in acyclic 9-month old animals compared to regular cyclers. Although expression level of other mitochondrial protein coding genes was not statistically different, they did share a trend of decreased gene expression. In terms of chronological aging, relative to 6-month-old female rats, mitochondrial genes in 9-month-old animals were generally up-regulated in hippocampus. However, following menopause, chronological aging did not affect mitochondrial gene expression. While the nuclear encoded genes exhibited a similar pattern of decline during perimenopause, a different pattern of expression was observed during chronological aging. Most of the nuclear encoded mitochondrial genes were up-regulated during chronological aging, especially after menopause when aging from 9 to 16 months. We also observed changes in key metabolic pathways. Our data suggest that in the hippocampus of aging female brain, mitochondrial and nuclear encoded electron transport chain subunits are under differential transcription regulation, and that the difference may be fuel source dependent.This work was supported by NIA 5P01AG026572 to RDB; Project 1 to RDB & EC.

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Poster

596. Brain Wellness and Aging: Models and Mechanisms

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Title: Physical and cognitive activity drive transcriptional signatures in the aged human hippocampus

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Abstract: A growing literature suggests that declines in cognitive performance associated with normal aging and even Alzheimer's disease (AD) can be buffered by physical and cognitive activity, with some evidence also supporting benefits of social participation. The underlying mechanisms by which lifestyle factors support brain function and health in the human brain are poorly understood.
We used a microarray-based genome-wide approach in combination with multiple linear regression analysis and high throughput data-mining tools to evaluate the relationship between hippocampal gene expression and late-life physical activity, cognitive frequency or social activity in the aged human brain. Hippocampal tissue was obtained from clinically well-defined cases that had been followed longitudinally for up to 18 years until death with detailed annual assessments of cognitive function and multiple lifestyle variables (physical activity, cognitive activity, social participation) as part of the Memory and Aging Project at Rush University Medical Center.
We found that physical and cognitive activity, but not social activity, have major programming effects on hippocampal gene expression patterns in the aged human brain and counteract specific age- and AD-related transcriptional patterns that contribute to cognitive decline. Core functions that are essential to build brain health are globally regulated at the transcriptional level, with physical and cognitive activity characterized by broadly increased expression of genes regulating mitochondrial function and energy production, protein trafficking and turnover, ubiquitin-proteasome function, RNA processing, DNA repair, and synaptic function. A central prediction of the study was that lifestyle modalities would counteract molecular changes specifically associated with aging and AD. Consistent with this hypothesis, our data revealed that 40% of the transcriptional changes associated with physical activity, and nearly 30% of those associated with cognitive activity, specifically counteracted Age- and AD-associated gene expression patterns in the human hippocampus. Our data are the first in the human brain to suggest that physical and cognitive activity promote at the transcriptional level a global shift toward preserved function of multiple core processes fundamental for maintaining a healthy brain state and high-level function with age.

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Poster

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Topic: C.01. Brain Wellness and Aging

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Title: Aging and exercise induced epigenetic modifications in prefrontal cortex of Wistar rats

Authors: *I. R. SIQUEIRA, C. G. BASSO, K. BERTOLDI, B. SCHALLENBERGER, L. C. MEIRELES, L. R. CECHINEL;
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Abstract: Beneficial effects of exercise have been recently linked to epigenetic mechanisms. Age and protocol- dependent epigenetic effects of exercise have been described in hippocampus of rats. However, the impact of treadmill exercise on epigenetic marks in aged cortices yet remains poorly understood. We investigated the effects of different exercise protocols, single session and daily exercise, on epigenetic marks, specifically H4 acetylation, DNA methytransferase (DNMT1 and DNMT3b), and histone methyltransferase H3K27 (HMT H3K27) activity, in prefrontal cortices from 3 and 21-months aged Wistar rats. The animals were submitted to two treadmill exercise protocols, single session or daily moderate. The daily exercise protocol induced an increased in histone H4 acetylation levels in prefrontal cortices of 21-months-old rats, without any effect in young adult group. DNMT3b levels were increased in aged cortices of animals submitted to single session of exercise. Our results indicate that prefrontal cortex is susceptible to epigenetic changes in a protocol and age dependent-manner and that H4 acetylation and DNMT3b changes might be linked at least in part to exercise-induced effects on brain functions in aging process.

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Poster

596. Brain Wellness and Aging: Models and Mechanisms

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Topic: C.01. Brain Wellness and Aging

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Title: Behavior, adult hippocampal neurogenesis & brain dopaminergic system in rat model with innate difference in running capacity & metabolism

Authors: *S. T. LENSU[1], M. S. NOKIA[2], E. MÄKINEN[1], H. VALLI[2], J. JOLKKONEN[3], L. G. KOCH[4], S. L. BRITTON[4,5], J. WIKGREN[2], H. S. O. KAINULAINEN[1];
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Abstract: Numerous studies have shown positive correlation between physical activity and brain health. Physical activity improves and maintains metabolic health, and seems to support learning and memory, and increase neurogenesis. However, it is difficult to make causal inferences between aerobic fitness and cognition, because exercise and a physically active lifestyle involve factors (e.g. social interaction and enriched living environment) known to support cognition. To study the neurobiological factors through which aerobic fitness influences cognition, we utilize a heterogenic rat model that is genetically determined for the intrinsic aerobic fitness: High and Low running Capacity Rats (HCR and LCR). Our group has shown that in this model, intrinsic aerobic fitness is related to improved learning that requires flexible cognition. All the experimental procedures were implemented in accordance with the directive 2010/63/EU of the European Parliament and approved by the National Animal Experiment Board, Finland. We determined baseline differences between the rat lines in spatial learning (T-maze test), brain dopaminergic system, and adult hippocampal neurogenesis (AHN). Both juvenile (at necropsy 8 weeks old) and adult rats (at necropsy 10 months old) were studied. AHN was studied by immunohistochemistry for doublecortin (protein expressed in newly divided cells maturing into neurons); brain dopaminergic system was investigated by immunohistochemistry for tyrosine hydroxylase (TH) and dopamine transporter. In behavior, no ratline differences were found in T-maze test at young ($p = 0.232$, mixed model ANOVA, LCR: $n = 19$, HCR: $n = 20$), while as an adult LCRs learned and remembered more efficiently this food motivated task compared with HCRs ($p < 0.001$, mixed model ANOVA, LCR: $n = 13$, HCR: $n = 12$). Young HCR rats ($n = 18$) had more new hippocampal neurons ($p < 0.05$, T-test) and more TH positive fibers ($p = 0.005$, t-test) in caudate putamen than LCRs ($n = 19$), while the analyses of adult animals are ongoing.

Our results indicate that a genetic predisposition for a high or a low aerobic capacity results differences within brain already as a young between LCR and HCR rats. The effect of aging remains to be shown. Furthermore, the studies for the effect of exercise on brain function and morphology would be needed, to see if we can diminish by exercise the early differences between the rat lines.

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Poster

596. Brain Wellness and Aging: Models and Mechanisms

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Topic: C.01. Brain Wellness and Aging

Support: Minnesota Women's Healthy Aging Project, University of Minnesota Foundation

American Legion Brain Sciences Chair, Univeristy of Minnesota

Title: Neural network decorrelation for healthy brain aging: A cross-sectional and longitudinal MEG study

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Abstract: Neural network decorrelation is regarded as fundamental to information processing. We have previously demonstrated that neural decorrelation, primarily involving temporal regions, distinguishes healthy veterans from those with psychiatric disorders, and have hypothesized that network decorrelation underlies healthy brain functioning by permitting neural flexibility (James et al., 2012 JAMA Psychiatry 70: 410-418). In the present study (http://brain.umn.edu/brp/womenshealthybrain.shtml), we evaluated neural network functioning derived from 1-min resting-state magnetoencephalographic (MEG; 248 sensors, sampled at 1.017 kHz) data in 185 cognitively healthy women, 30-100+ years old, who have participated in a longitudinal study aimed at defining factors underlying healthy brain function across the lifespan. We expected that within and among cognitively healthy individuals, decorrelation would increase with age, serving as a mechanism that promotes neural flexibility and maintains

healthy brain functioning across the lifespan. To test that hypothesis, we determined the strength of network functional connectivity by computing all pairwise crosscorrelations (-50 to +50 lags, 0.974 ms/lag) between prewhitened MEG sensor time-series and regressing them against age (in months) to evaluate neural network properties cross-sectionally and longitudinally. As expected, results suggested that among cognitively healthy women, decorrelation increased with age both cross-sectionally and longitudinally, and involved primarily the temporal lobes, within and across hemispheres. Furthermore, the amount and location of decorrelation varied across lags, providing robust real-time evidence of the active neural processes involved in decorrelation. These findings support our hypothesis above that neural network decorrelation underlies healthy brain functioning by maintaining adequate capacity of the networks for information processing.

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Poster

596. Brain Wellness and Aging: Models and Mechanisms

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 596.17/M9

Topic: C.01. Brain Wellness and Aging

Support: USDA Intramural

Washington Red Raspberry Commission

Title: A clinically relevant frailty index for aging rats

Authors: *M. G. MILLER[1], N. THANGTHAENG[2], T. M. SCOTT[2], B. SHUKITT-HALE[2]; [1]Neurosci. and Aging Lab., [2]USDA-HNRCA, Boston, MA

Abstract: Frailty is a clinical syndrome that is increasingly prevalent during aging. Frailty involves the confluence of reduced strength, speed, physical activity, and endurance, and it is associated with adverse health outcomes. Frailty indices have been developed to diagnose frailty in older adult populations, and a pre-clinical frailty index has been developed for the C57BL/6 mouse. The present study adapts these existing clinical and pre-clinical indices of frailty to the Fischer (F344) rat, which is commonly used in aging research. One hundred and thirty-three male F344 rats (17mo) completed a battery of commonly administered behavioral tasks, including: forelimb wire-hang (strength), rotarod (speed), open field (physical activity), and inclined screen (endurance). This age was selected because it provides adequate remaining lifespan in which to conduct an intervention study without undo mortality and at which age-

related risk factors may still be modifiable. Within this cohort, rats whose performance put them in the lowest quintile were identified for each task, and the number of tasks on which a rat performed poorly determined their frailty index. Rats that performed poorly on two tasks were considered mildly frail (17.29%, n = 23), and rats that performed poorly on 3-4 tasks were considered frail (2.26%, n = 3). Logistic regression of 100-day survival revealed that mildly frail rats were 3.8 times and frail rats were 27.5 times more likely to die during that period than non-frail rats (p = 0.038; 95% CI: 2.030, 372.564). The selected criterion tests, cutoff points, and index provide a potential standardized definition for frailty in aged F344 rats that is consistent with existing frailty indices for humans and mice, and present a potentially useful tool for excluding frail animals which may not survive a chronic intervention study.

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Poster

596. Brain Wellness and Aging: Models and Mechanisms

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Topic: C.01. Brain Wellness and Aging

Support: USDA Intramural

Washington Red Raspberry Commission

Title: Raspberry supplementation alleviates age-related motor dysfunction in select populations

Authors: ***N. THANGTHAENG**, M. G. MILLER, M. E. KELLY, D. E. SMITH, B. SHUKITT-HALE;
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Abstract: Age-related declines in balance, muscle strength and coordination often lead to a higher incidence of falling. Among older adults, falls are the leading cause of distress, pain, injury, loss of confidence, and ultimately, loss of independence and death. Previous studies in our laboratory have demonstrated that berry supplementation improves age-related declines in motor function even when fed to aged animals. However, it is still unclear whether the requisite daily intake for efficacy differs based on motor capability. The purpose of this study was to explore the interaction between baseline motor performance and daily raspberry intake required to achieve improvement and/or preservation in motor function. Aged male F344 (17 mos old) rats were tested for baseline (pre-test) balance, muscle strength and coordination and divided into

good, average and poor performers based on their motor composite score (MCS), which was comprised of tests for strength (grip strength, wire suspension, and inclined screen), balance (plank and rod walking) and endurance (accelerating rotarod). Rats in each category were fed with either a 0%, 1%, or 2% raspberry-supplemented diet (RB). After 8-weeks on their respective diets, the rats were retested (post-test). Overall, the good performers performed worse in all of the post-tests compared to the pre-tests regardless of their diet. Interestingly, 1% and 2% RB appeared to preserve the performance of the good performers and improve the performance of poor performers on plank walking ($p< 0.05$). Two-percent RB improved post-test grip strength of the poor performers ($p<0.05$). Poor performers fed with 1% or 2% RB had higher post-test MCS ($p< 0.05$), while 2% RB lowered post-test MCS in the good performers ($p< 0.05$). The findings from this study identified poor performers as being most likely to benefit from daily consumption of ½-1 cup of raspberry to improve/preserve motor function. When extended to humans, raspberry may reduce fall risk, extend independence, and improve quality of life in the aging population.

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Poster

596. Brain Wellness and Aging: Models and Mechanisms

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Topic: C.01. Brain Wellness and Aging

Support: USDA Intramural

U.S. Highbush Blueberry Council

California Strawberry Commission

Title: The effects of blueberry and strawberry serum metabolites on age-related oxidative and inflammatory stress signaling *In vitro*

Authors: D. R. FISHER[1], M. G. MILLER[1], N. THANGTHAENG[1], M. E. KELLY[1], D. F. BIELINSKI[1], *B. SHUKITT-HALE[2];
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Abstract: Age-related decrements in cognition are thought to result from the increased susceptibility to and accumulating effects of oxidative stress and inflammation. Berry fruits contain a variety of bioactive polyphenolic compounds, such as anthocyanins, that exhibit potent

antioxidant and anti-inflammatory activities. In previous studies, we have shown that consumption of freeze-dried whole berry powder, equivalent to 1 cup/day of blueberry (BB) or 2 cups/day of strawberry (SB), can differentially improve some aspects of cognition in healthy, older adults, compared to placebo-supplemented controls. Cell models provide valuable tools for the development of novel strategies and assessment of the mechanisms behind the protective effects of various foods against the oxidative stress and inflammation seen in aging. Because the bioactive compounds in foods are different than those found in circulation following consumption, pre-treatment of cells with serum from people fed these foods may be a more valid model system than treating with extracts of the foods themselves. In this study, we investigated whether fasting and postprandial serum from BB- or SB-supplemented older adults (60-75yo) taken at baseline or after 45 or 90 days of supplementation would reduce the production of inflammatory and oxidative stress signals, compared to a placebo group, in stressed HAPI rat microglial cells, *in vitro*. Serum from both blueberry and strawberry were able to reduce inflammatory stress signals, e.g. nitric oxide ($p < 0.05$), relative to serum from placebo controls. Measurements of oxidative stress signaling are currently ongoing. These results suggest that berry metabolites, present in the circulating blood, may be mediating the anti-inflammatory effects of dietary berry fruit. It is likely that this attenuation of inflammation is responsible for the beneficial effects on age-related declines in cognition.

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Poster

597. Mitochondria and Energy Metabolism in Health and Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R21 NS 090243

R21 NS084156

National Parkinson’s Foundation

R01 AG046396

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P50 AG00513421

Title: Tau PET imaging in the Lewy body diseases

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Abstract: Background The causes of cognitive impairment in dementia with Lewy bodies (DLB) and Parkinson disease (PD) are multifactorial. Tau pathology is commonly observed at autopsy in DLB and PD dementia, but its contribution during life to these diseases is unknown. In this study, we sought to contrast tau aggregation in DLB, cognitively impaired PD (PD-impaired), cognitively normal PD (PD-normal), and normal control (NC) subjects and to evaluate the relationship between tau aggregation, amyloid deposition, and cognitive function. **Methods** This cross-sectional study was conducted from 2014 to 2016. Subjects were recruited from the MGH Memory and Movement Disorders Units. 24 patients with Lewy body disease (7 DLB, 8 PD-impaired, and 9 PD-normal) underwent multimodal brain imaging, cognitive testing, and neurological evaluation, and imaging measures were compared to those of an independently acquired group of 29 NC subjects with minimal brain amyloid burden, as measured with [^{11}C]PiB PET. Subjects underwent tau PET imaging with [^{18}F]AV-1451, MRI, detailed cognitive testing, and neurological examination. All but 3 subjects also underwent amyloid imaging with [^{11}C]PiB PET. **Results** In DLB, cortical [^{18}F]AV-1451 uptake was highly variable and greater than in NC, particularly in the inferior temporal gyrus (ITG) and precuneus. Foci of increased [^{18}F]AV-1451 binding in the ITG and precuneus were also evident in PD-impaired subjects. Elevated cortical [^{18}F]AV-1451 binding was observed in 4/17 Lewy body disease cases with low cortical [^{11}C]PiB retention. For DLB and PD-impaired subjects, greater [^{18}F]AV-1451 uptake in the ITG and precuneus was associated with increased cognitive impairment, as measured with the MMSE and the CDR sum-of-boxes score. **Conclusions** Patients with Lewy body disease manifest a spectrum of tau pathology. Cortical aggregates of tau are common in DLB and PD-impaired patients, even in patients without elevated amyloid. When present, tau deposition is associated with cognitive impairment. These findings support a role for tau co-pathology in the Lewy body diseases.

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Poster

597. Mitochondria and Energy Metabolism in Health and Disease

Location: Halls B-H

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Program#/Poster#: 597.02/M13

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant 3R01GM109434-01A1S1

Title: Elucidating the relationship between mitochondrial respiratory supercomplex plasticity and reactive oxygen species production

Authors: *C. A.-M. PORRAS[1], Y. BAI[2];
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Abstract: The textbook model of the mitochondrial respiratory chain describes the system as a liquid arrangement of respiratory complexes acting as independent entities. Inversely the solid model envisions these complexes coming together to form stable, functional, supra-molecular supercomplexes. New evidence however points to a more dynamic environment between these two extremes. The plasticity model describes a cell that is able to manipulate the composition of its respiratory chain to favor certain confirmations of complexes in response to changes in its environment. In this model the more efficient supercomplexes are especially important for cell types with a high ATP demand as a method for preventing reactive oxygen species (ROS) production. In contrast cells with a low ATP demand may prefer to use free complexes to avoid the extra energy demand to build and maintain supercomplexes.
In this study we have attempted to observe supercomplex plasticity by using serum-starvation to induce a shift in the balance between supercomplexes and free complexes. In this situation growth factors are removed, and the cells become quiescent and thus lower their ATP demand, so according to the plasticity model there should be a shift away from supercomplexes towards free complexes. Using Blue Native Gel Electrophoresis, we have shown that this shift does occur during chronic serum-starvation. Additionally during this shift in supercomplex assembly, there is a decrease in ATP production and a robust increase in ROS production. This increase may hint at the role of supercomplexes in maintaining a low level of ROS by an efficient mitochondrial respiratory chain.
This role has far reaching applications in diseases characterized by oxidative stress. In particular supercomplexes may play a relevant role in neurodegenerative disease and the aging process where the ability to build supercomplexes may be lost in neurons that are sensitive to mitochondrial dysfunction. In these cell types, the demand for ATP and the loss of supercomplexes may lead to rampant ROS production causing extensive damage to proteins, lipids, and DNA. In aging this may cause a vicious cycle where oxidative damage causes the

respiratory complexes to become even more inefficient leading to further production of ROS. This research helps to establish a system whereby supercomplex plasticity and the mechanisms that regulate it can be studied in an effort to discover therapeutic targets that can rescue the loss of supercomplexes and potentially reduce the production of ROS.

Disclosures: **C.A. Porras:** None. **Y. Bai:** None.

Poster

597. Mitochondria and Energy Metabolism in Health and Disease

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Support: NSC 102-2314-B-038-002-MY2

MOST-103-2314-b-038-024-

TMU101-AE1-B37

Title: CCL5/RANTEs contributes to the hypothalamic insulin sensitivity to systemic insulin sensitivity through CCR5

Authors: ***S.-Y. CHOU**, Y.-T. HSIEH, R. AJOY;
Grad. Inst. of Neural Regenerative Med., Taipei Med. Univ., Taipei, Taiwan

Abstract: Many neurodegenerative diseases are accompanied by impaired energy metabolism as also seen with as diabetes mellitus (DM). CCL5/RANTES and its receptor CCR5 are known to contribute to neuronal activity a as well as type 2 DM but their mechanisms are unclear. Herein, we found that the lack of CCR5 or CCL5 in mice impaired the regulation of energy metabolism in hypothalamus. Immunostaining and co-immunoprecipitation revealed the specific expression of CCR5, associated with insulin receptors, in hypothalamic arcuate nucleus (ARC). Both ex vivo stimulation and in vitro culture studies demonstrated that the activation of insulin, and PI3K-Akt pathway were impaired in CCR5 and CCL5 deficient hypothalamus. The inhibitory phosphorylation of insulin response substrate-1 (IRS-1) at Ser302 (IRS-1S302) by insulin was markedly increased in CCR5 and CCL5 deficient animals but not IRS-2. Elevating CCR5 by CCL5 induced GLUT4 membrane translocation and reduced phospho-IRS-1S302 through AMPKα - S6 Kinase. Blocking CCR5 using the antagonist, MetCCL5, abolished the de-phosphorylation of IRS-1S302 and insulin signal activation. In addition, intracerebroventricular delivery of MetCCL5 interrupted hypothalamic insulin signaling and elicited peripheral insulin responsiveness and glucose intolerance. Taken together, our data suggest that CCR5 regulates

insulin signaling in hypothalamus which contributes to systemic insulin sensitivity and glucose metabolism.

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Poster

597. Mitochondria and Energy Metabolism in Health and Disease

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Program#/Poster#: 597.04/M15

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AbbVie

Title: *In vivo* evaluation of cerebral glucose utilization in aged rats and non-human primates assessed by [^{18}f]-fluorodeoxyglucose positron emission tomography

Authors: ***A. M. BASSO**, R. RAJAGOVINDAN, D. R. REUTER, A. E. TOVCIMAK, S. J. BAKER, B. A. HOOKER, M. J. VOORBACH, J. D. BEAVER;
Translational Sciences-Imaging, AbbVie, North Chicago, IL

Abstract: Alzheimer's disease (AD) is associated with reduction in glucose utilization in temporal, posterior cingulate, frontal cortex and hippocampal brain areas during early asymptomatic stages of the disease, in patients with mild cognitive impairment and in individuals at risk for AD. The progressive decrease of cerebral metabolic rate of glucose is related to the severity of cognitive and functional decline in AD. Reduction in cerebral glucose uptake assessed by [^{18}F]-fluorodeoxyglucose positron emission tomography (FDG PET) has been proposed as a sensitive pharmacodynamic marker to quantify physiologic /metabolic processes in the brain *in vivo*, associated with neurodegenerative pathologies and potential modulation by novel therapies. The aim of the current study was to test the hypothesis that cerebral glucose uptake *in vivo* in aged animals (rats and non-human primates, NHP) is reduced compared to young control subjects and can serve as a suitable preclinical model of impaired cerebral metabolic function. Male Sprague Dawley rats (5-7 month-old, n=23 and 20 month-old, n=19) and female cynomolgus macaques (5-7 year-old, n= 7 and 20-21 year-old, n=7) were evaluated for brain glucose utilization using the Inveon PET/CT scanner (Siemens) and MicroPET Focus 220 (Siemens). Animals were fasted overnight (16-18 h) and plasma glucose levels were measured prior to each scan to ensure eligibility of the animal for the study. In all cases, [^{18}F]-FDG was injected i.v. into awake animals and after approximately 45-50 min, they were anesthetized (1.75-2.5% isoflurane) for CT followed by a static PET scan for 10 or 30 min duration (rats and NHP respectively). The average interval between [^{18}F]-FDG administration

and PET scan was 73+/-8 min in NHP. Throughout the study, body temperature of the animals was maintained stable. Standardized uptake values (SUV) in brain regions of interest were derived followed by statistical analysis employing linear mixed effect model with Group (young, aged) as a fixed factor and plasma glucose level as a continuous covariate. [^{18}F]-FDG whole brain SUV glucose uptake in aged rats was significantly decreased (approximately 35% reduction) compared to young adults ($p<0.01$). In the NHP study, there was also an effect of age, with older animals demonstrating 20-25% reduction in FDG brain uptake (SUV) relative to the young animals in the hippocampus, posterior cingulate, temporal cortex, occipital cortex , frontal cortex and cerebellum ($p<0.05$). Results confirm our hypothesis and highlight the potential value of using aged animals to study brain metabolic changes as a preclinical model relevant for aging, AD and neurodegenerative diseases.

Disclosures: **A.M. Basso:** A. Employment/Salary (full or part-time): AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie. **R. Rajagovindan:** A. Employment/Salary (full or part-time): AbbVie. **D.R. Reuter:** A. Employment/Salary (full or part-time): AbbVie. **A.E. Tovcimak:** A. Employment/Salary (full or part-time): AbbVie. **S.J. Baker:** A. Employment/Salary (full or part-time): AbbVie. **B.A. Hooker:** A. Employment/Salary (full or part-time): AbbVie. **M.J. Voorbach:** A. Employment/Salary (full or part-time): AbbVie. **J.D. Beaver:** A. Employment/Salary (full or part-time): AbbVie.

Poster

597. Mitochondria and Energy Metabolism in Health and Disease

Location: Halls B-H

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Program#/Poster#: 597.05/M16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Marga and Walter Boll Foundation

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Title: Comparing arterial spin labeling-based brain perfusion to gray matter probability and glucose metabolism in patients with Alzheimer’s disease and subjective cognitive decline

Authors: ***N. NELLESSEN**[1], N. RICHTER[2], K. ZHANG[4], K. N. H. DILLEN[5], H. I. L. JACOBS[7,8,9], B. VON REUTERN[2,5], Ö. A. ONUR[3,5], G. STOFFELS[6], E. ROTA KOPS[6], L. TELLMANN[6], N. J. SHAH[6], K. J. LANGEN[6,10], G. R. FINK[2,5], J. KUKOLJA[2,5];
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Univ., Cologn, Germany; [4]Dept. of Radiology, German Cancer Res. Center-DKFZ, Heidelberg, Germany; [5]Cognitive Neuroscience, Inst. of Neurosci. and Med. (INM-3), [6]Inst. of Neurosci. and Med. (INM-4), Res. Ctr. Jülich, Jülich, Germany; [7]Fac. of Health, Med. and Life Sciences, Sch. for Mental Hlth. and Neurosci., [8]Dept. of Psychiatry and Neuropsychology, Maastricht Univ., Maastricht, Netherlands; [9]Dept. of Radiology, Athinoula A. Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA; [10]Dept. of Nuclear Med., Univ. of Aachen, Aachen, Germany

Abstract: Background: Pseudo-continuous arterial spin labeling (pCASL) is a magnetic resonance imaging (MRI) technique for contrast agent-free perfusion measurement. Previous studies found associations between pCASL with fluorodeoxyglucose positron emission tomography (FDG-PET).
Goals: We aimed at comparing perfusion (pCASL) to the two traditional imaging methods of voxel based morphometry for measurement of gray matter probability (GM) and quantification of glucose metabolism (FDG-PET). We examined patients with subjective cognitive decline (SCD) and manifest Alzheimer's disease (AD).
Methods: 19 SCD (aged 65.3 ± 8.3, MMSE 28.6 ± 2.0) and 25 AD patients (aged 70.4 ± 6.0, MMSE 24.3 ± 2.7) were scanned with pCASL, T1 mprage and FDG-PET (N: 11 SCD & 17 AD). All statistical tests were age- and atrophy-corrected, if applicable, and restricted to a region of interest encompassing the parietal cortex and (para-)hippocampus. Analyses of co-variance (ANCOVA) tested for between-group differences of mean GM, mean glucose metabolism and mean perfusion with eta squared (η^2) as the corresponding effect size. Furthermore, a voxel-wise regression evaluated regional distribution of group differences of perfusion. Mean GM, mean glucose metabolism and mean perfusion in AD were correlated with each other in a pairwise manner.
Results: Mean GM, glucose metabolism and perfusion were reduced in AD compared to SCD ($p<.0000005$, $\eta^2 =.35$, $p<.00001$, $\eta^2 =.15$; $p<.05$, $\eta^2 =.01$, respectively). The voxel-wise regression revealed lower perfusion in the posterior cingulate cortex (PCC) in AD compared to SCD ($p<.05$, family wise error-corrected). Glucose metabolism correlated with perfusion in AD ($p<.05$, $r=.51$).
Discussion: All measures were significantly reduced in AD. GM was reduced with a large effect, and after age and atrophy correction, glucose metabolism with a medium and perfusion only with a small effect. Perfusion was especially lower in the bilateral PCC in AD compared to SCD. Functional measures (perfusion and glucose metabolism) were associated with each other in AD, while neither was with structural measures (GM). FDG-PET and perfusion shared similar informative values, while FDG-PET and GM were much more robust in discriminating AD and SCD. Further research is warranted to aid in establishing pCASL as both, an addition and, in some cases, as an alternative to PET imaging.

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Poster

597. Mitochondria and Energy Metabolism in Health and Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Comparison of hypometabolism and cortical atrophy in Primary Progressive Aphasia

Authors: *K. ADAMCZUK[1,2,3,4], M. STEPANOVIC[1,3], S. MAKARETZ[1,5], M. BRICKHOUSE[5,3], C. CASO[5,6], M. QUIMBY[5], R. VANDENBERGHE[2,7], B. DICKERSON[5,1,3,4]; [1]MGH Dept. of Neurol., Boston, MA; [2]KU Leuven, Leuven, Belgium; [3]Martinos Ctr. for Biomed. Imaging, Boston, MA; [4]Harvard Med. Sch., Boston, MA; [5]MGH Frontotemporal Disorders Unit, Boston, MA; [6]MGH Dept. of Psychiatry, Boston, MA; [7]UZ Leuven Dept. of Neurol., Leuven, Belgium

Abstract: BACKGROUND: Recent diagnostic criteria for Primary Progressive Aphasia (PPA) include neuroimaging as a supportive feature [Gorno-Tempini et al., 2011]. The regional overlap between atrophy (measured by structural MRI) and hypometabolism (measured by FDG-PET) may vary throughout the PPA spectrum. Comparison of territories affected by atrophy and hypometabolism will shed light on the sequence of pathophysiological changes in the disease.
HYPOTHESIS: Subtle hypometabolism is detectable in the PPA affected language regions which do not yet show cortical atrophy.
METHODS: 30 right-handed PPA patients (10 logopenic, 11 non-fluent agrammatic, 9 semantic) underwent FDG-PET scan within 12 months from structural MRI scan. PET was co-registered to MRI and glucose uptake was quantified by standardized uptake value ratios (SUVR) with pons as reference region. MRI scans were analyzed according to FreeSurfer pipeline. SUVR and cortical thickness values were projected to surfaces in fsaverage space. Hypometabolism and atrophy Z-maps were calculated based on a set of 30 age and sex matched controls from Harvard Aging Brain Study [Dagley et al., 2015].
RESULTS: Hypometabolism and cortical atrophy were localized to regions involved in language dysfunction in PPA, which were mainly left hemispheric. Regions with cortical atrophy were smaller and contained within regions displaying hypometabolism.
CONCLUSION: Our data suggest that hypometabolism is present without co-occurring atrophy in parts of the PPA affected language regions, which likely indicate future disease spread. Hypometabolism reflects synaptic and neuronal dysfunction, therefore, may occur earlier than cortical atrophy which represents neuronal death.

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Poster

597. Mitochondria and Energy Metabolism in Health and Disease

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Topic: C.01. Brain Wellness and Aging

Support: HD 080910

Title: Evaluating a lack of creatine in the dopaminergic neurotransmitter system

Authors: *Z. I. ABDULLA[1,2], B. PAHLEVANI[1], M. SKELTON[1,2];
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Abstract: Creatine's (Cr) primary function is as an intracellular buffer that maintains high levels of ATP through the Cr/Phosphocreatine (PCr) shuttle. In this reversible reaction PCr donates its phosphate group to ADP, yielding ATP. This is relevant to the pathology associated with Parkinson's disease (PD) as reductions in both ATP and PCr are observed in the dopamine (DA) rich midbrain, caudate, and putamen of those with PD. Cr also exhibits mitochondria-protective properties and dysfunctional mitochondria have been observed in the substantia nigra of those with PD. Importantly, Cr relies upon the Na^+, Cl^--dependent Cr transporter (CrT) to enter cells. The CrT is encoded by the X-linked SLC6A8 gene, mutations of which result in CrT Deficiency (CTD), a developmental disorder characterized by a lack of cellular creatine, intellectual disability, epilepsy, and autistic-like symptoms. A high comorbidity of attention-deficit hyperactivity disorder (ADHD) - itself often attributed to dysfunctions in DA modulation - is also reported. With this in mind, we evaluated adult mice lacking Cr in the DA neurotransmitter system for PD-like symptoms and hyperactivity. Dopamine-specific *Crt* knockout (DAT-CrT) mice were generated by crossing $Crt^{flox/+}$ mice with mice that express Cre recombinase driven by the DA transporter (DAT) promoter. Brain specific *Crt* knockout (BKO) and $Crt^{flox/y}$ mice were included as controls. Motoric function was evaluated every 30 days from P90 until P360. Locomotor activity was evaluated in both light and dark phases. In the challenging beam (CB) task, mice traverse a beam consisting of four 25 cm segments, each segment being 1 cm narrower than the previous. Errors, latency to transverse the beam and steps taken are recorded. Spontaneous activity (SA) was assessed by placing mice in a glass beaker on a transparent table and recording number of fore and hind limb steps, total rears and grooming events, and time spent grooming. Gait was analyzed using the DigiGait system. No differences were observed in measures indicative of a parkinsonian phenotype, i.e. increased errors in CB or decreased activity in SA. Both DAT-CrT and BKO mice display hyperactivity, evidenced by their quickness to cross the beam in CB as well as increased locomotion during both light and dark phases. The hyperactivity in DAT-CrT mice suggests that depleting Cr in DA neurons results in

hyperactivity. Furthermore, this could point to energetic impairments in the DA system as a mechanistic factor of ADHD in CTD.

Disclosures: Z.I. Abdulla: None. **B. Pahlevani:** None. **M. Skelton:** None.

Poster

597. Mitochondria and Energy Metabolism in Health and Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 597.08/N1

Topic: C.01. Brain Wellness and Aging

Support: Le Foundation

partial support by grant # UL1 TR000043 from the National Center for Research Resources and the National Center for Advancing Translational Sciences (NCATS).

Title: Sleep disordered breathing severity is related to GABA in the Dorsolateral Prefrontal Cortex

Authors: *A. C. PEREIRA[1], R. DAVIDSON[2], A. KRIEGER[4], D. SHUNGU[4], B. MCEWEN[3]; [1]Neuroscience/ McEwen laboratory, [2]Rockefeller Univ., New York, NY; [3]Rockefeller Univ., Rockefeller University, NY; [4]Weill Cornell Med. Col., Weill Cornell Medical College, NY

Abstract: Sleep-disordered breathing (SDB), a condition with repeated episodes of hypopnea and apnea during sleep, is more prevalent in the elderly than younger populations, and can contribute to cognitive decline. SDB is characterized by sleep fragmentation and intermittent hypoxia. Hypoxia in animal models have resulted in dysregulation in gamma-aminobutyric acid (GABA) and glutamate, the major inhibitory and excitatory neurotransmitters in the brain, respectively, leading to excitotoxicity, neuronal damage and/or loss. However, excitatory and inhibitory neurotransmitter dysregulations in the human elderly brain with SDB remain poorly understood, and in particular in the dorsolateral prefrontal cortex (dlPFC). The dlPFC is critical for executive functions and it is highly vulnerable to synaptic and functional changes in aging. SDB can exarcebate executive dysfunction and other cognitive impairment in the elderly, decreasing quality of life. Investigation of the neurometabolic changes in elderly SDB patients can advance our understanding of the neuropathophysiology of SDB and point to potential novel biomarkers and treatment targets. We used proton magnetic resonance spectroscopy (H MRS) which allows *in vivo* non-invasive measures of neurometabolites such as GABA and Glx (combines resonances of glutamate and glutamine) in the dlPFC and in the hippocampus of elderly individuals with moderate-severe SDB (AHI>15) and without SDB (AHI<5), who have

undergone polysomnography, to examine the hypothesis that alterations in one or both of these neurotransmitters occur in SDB and correlates with oxygenation measures, potentially contributing to increased neural excitability in SDB. GABA levels were significantly reduced in the dlPFC in elderly with SDB compared with elderly subjects without SDB. Moreover, we observed a relationship between GABA levels in dlPFC and severity of sleep apnea, measured through apnea-hypoxia index (AIH) and minimal saturation levels. Our findings implicating GABA in the pathophysiogical mechanisms of SDB may contribute to future studies that can ultimately help prevent neural damage and cognitive decline in the elderly population and identify novel treatment targets.

Disclosures: **A.C. Pereira:** None. **R. Davidson:** None. **A. Krieger:** None. **D. Shungu:** None. **B. McEwen:** None.

Poster

597. Mitochondria and Energy Metabolism in Health and Disease

Location: Halls B-H

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Program#/Poster#: 597.09/N2

Topic: B.05. Transporters

Support: DFG He1128/18-1

DFG EXC 257

Title: 4-CIN, a neuronal lactate inhibitor affects ion homeostasis and energy metabolism

Authors: ***U. HEINEMANN**[1], R. KOVACS[1], E. A. ANGAMO[2];
[1]Neurosci. Res. Ctr., Charité Universitätsmedizin Berlin, Berlin, Germany; [2]Neurosci. Res. Ctr., Charite Universitätsmedizin Berlin, Berlin, Germany

Abstract: Here, we determined the contribution of astrocytic neuronal lactate shuttle to maintenance of ion homeostasis and energy metabolism. We tested for the effects of α-cyano-4-hydroxycinnamic acid (4-CIN, less tan 200 μM), which could interfere with energy metabolism by blocking monocarboxylate-transporter 2 (MCT2) mediated neuronal lactate uptake, on evoked potentials, stimulus induced changes in K^+, Na^+, Ca^{2+}, pH and oxygen concentrations as well as on changes in flavine-adenine dinucleotide (FAD) autofluorescence in hippocampal area CA3. MCT blockade by 4-CIN reduced synaptically evoked but not antidromic population spikes. This effect was dependent on the activation of K_{ATP} channels indicating reduced neuronal ATP synthesis. By contrast, lactate receptor activation by 3,5-dihydroxybenzoic acid (3,5-DHBA) resulted in increased antidromic and orthodromic population spikes suggesting that 4-

CIN effects are not mediated by lactate accumulation and subsequent activation of lactate receptors. Recovery kinetics of all ion transients were prolonged and baseline K^+ concentration and proton concentration became elevated by blockade of lactate uptake. Lactate contributed to oxidative metabolism as both baseline respiration and stimulus induced changes in pO_2 were decreased, while FAD fluorescence increased likely due to a reduced conversion of FAD into $FADH_2$. These data suggest that lactate shuttle contributes to regulation of ion homeostatsis and synaptic signaling even in the presence of ample glucose.

Disclosures: **U. Heinemann:** None. **R. Kovacs:** None. **E.A. Angamo:** None.

Poster

597. Mitochondria and Energy Metabolism in Health and Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 597.10/N3

Topic: B.10. Network Interactions

Title: Nrf1 knockdown as a modulator of mitochondrial electron transport chain and biogenesis

Authors: ***P. SADEGHI**[1,2], S. KHALIFEH[3,4], F. KHODAGHOLI[2,1], M.-R. ZARRINDAST[3,4]; [1]Neurosci. Res. Ctr., Tehran, Iran, Islamic Republic of; [2]NeuroBiology Res. Ctr., Shahid Beheshti Univ. of Med. Sci., Tehran, Iran, Islamic Republic of; [3]Med. Genomics Res. Ctr. and Sch. of Advanced Sci. in Med., [4]Cognition and neuroscience research center, Islamic Azad University, Tehran Med. Sci. Br., Tehran, Iran, Islamic Republic of

Abstract: ***Introduction***
The nuclear factor, erythroid-derived 2,-like 1(Nrf1) is a member of the cap "n" collar subfamily of basic region leucine zipper transcription factors and plays major role in regulating the adaptive response to oxidants and electrophiles within the cell. Interestingly, mitochondrial electron transport chain (ETC) is the most energy producer in the cell which consists of multi-subunit enzyme complexes. Mitochondrial biogenesis results from organized and precise activity of nuclear and mitochondrial genomes. In the present study, the level of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), an important factor involved in mitochondrial biogenesis has been evaluated after Nrf1-siRNA injection.
Methods
Here, we injected small interfering RNA (siRNA) targeting Nrf1 in dorsal third ventricle (D3V) of adult male albino Wistar rats (weighting 200-250 g) and subsequently examined the effect of this silencing on mitochondrial biogenesis factors, along with ETC activity in three brain regions: hippocampus, amygdala, and prefrontal cortex. Three groups were designed for this study: control group 1 received intra-D3V injection of 5 µl scrambled siRNA; control group 2

received 5 µl RNase-free water in their D3V, and the third group received intra-D3V injection of 5 µl Nrf1-siRNA. Stereotaxic surgery and siRNA administration in rat brain was conducted due to standard protocol. To be noticed, western blotting using Anti-Nrf1 and PGC-1α antibodies and spectrophotometric analysis of ETC complexes activities were applied for evaluation.

Results

We evaluated the level of Nrf1 by Western blotting, 4 and 8 h after Nrf1-siRNA injection. Protein level of Nrf1 reduced in consequence of Nrf1-siRNA injection. The most reduction of Nrf1 level was detected in prefrontal cortex, 8 h after siRNA injection (35.5 % compared to the control group). Also, having ETC as the most energy producer in the cells, complex I activity did not show any significant changes in all studied regions. Complexes II-III activity did not change significantly in prefrontal cortex and amygdala, but accumulated activity of complexes II-III in hippocampus increased to 32.13 and 33.76 nmol/min/mg protein, 4 and 8 h after Nrf1-siRNA knockdown, respectively. Otherwise, activity of complex IV in all three regions increased significantly. In addition, Nrf1-siRNA injection increased PGC-1α and cytochrome-c level compared to the control group. The most increase in PGC-1α level (2.3-fold compared to the control group) was in amygdala, 8 h after Nrf1-siRNA injection.

Disclosures: P. Sadeghi: None. **S. Khalifeh:** None. **F. Khodagholi:** None. **M. Zarrindast:** None.

Poster

597. Mitochondria and Energy Metabolism in Health and Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 597.11/N4

Topic: H.01. Animal Cognition and Behavior

Title: Changes in the content of GFAP and MAP-2 in the hippocampus of the rats with offspring female of mothers fed low-protein diets in pregnancy and/or lactation.

Authors: *R. B. GARCIA[1], C. T. SOSA-LARIOS[2], T. NERI-GOMEZ[3], A. E. GOMEZ-MARTÍNEZ[4], A. C. MENDOZA-REYES[5], L. S. MORIMOTO-MARTÍNEZ[2];
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Abstract: The fetal and neonatal pancreas shows developmental plasticity and responsiveness to its metabolic environment, including poor maternal nutrition. Low-protein (LP) maternal diets

decrease fetal b-cell mass and isolated islet insulin secretion at term. Life-time consequences of poor fetal pancreatic development can impair offspring carbohydrate metabolism predisposing to diabetes even when the diet is normalized at weaning. Also this can leads to damage in the brain since the developmental stage in which nutrition was altered. The effect can alter in short term some neural process such learning and memory, anxiety, satiety, oxidative stress causing in an long term a development of neurodegenerative disease like Alzheimer. The aim for this work was to study the effect of the low protein diets in pregnancy and/or lactation rats on the hippocampus, a special area for memory and learning. We studied female rat offspring exposed to low-protein maternal diet (50% control protein diet) in pregnancy and/or lactation at postnatal day 36. Rats were fed either control 20% casein diet (C) or restricted diet (R - 10% casein) during pregnancy. After delivery, mothers received either C or R diet until weaning to provide four offspring groups: CC, RR, CR and RC (first letter denoting maternal pregnancy diet and the second lactation diet). Pups were euthanized at 36 days old and the hippocampus was dissected and western blot was performed to evaluate the context of GFAP and MAP-2. For GFAP was observed an increase in the RR and RC diets, on the other hand for MAP-2 a decrease in the diets RR and RC. This result suggest that a restriction diet and a diet that after is normalized affect some molecular process in the brain and this can leads to an behavioral impairment at long term.

Disclosures: **R.B. Garcia:** A. Employment/Salary (full or part-time): UNAM. **C.T. Sosa-Larios:** A. Employment/Salary (full or part-time): INNCMNSZ. **T. Neri-gomez:** A. Employment/Salary (full or part-time): IMSS. **A.E. Gomez-martínez:** A. Employment/Salary (full or part-time): UNAM. **A.C. Mendoza-reyes:** A. Employment/Salary (full or part-time): UNAM. **L.S. Morimoto-Martínez:** A. Employment/Salary (full or part-time): INNCMNSZ.

Poster

597. Mitochondria and Energy Metabolism in Health and Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 597.12/N5

Topic: A.01. Neurogenesis and Gliogenesis

Support: E0752801

Title: Acetyl l-carnitine targets ATP synthase in protecting zebrafish embryos from ketamine-induced toxicities

Authors: ***J. KANUNGO**, B. ROBINSON, Q. GU, S. ALI, M. PAULE, X. GUO; Neurotoxicology, Natl. Ctr. For Toxicological Research/Food and Drug Admin., Jefferson, AR

Abstract: Ketamine, an antagonist of the Ca^{2+}-permeable N-methyl-d-aspartate (NMDA)-type glutamate receptors, is a pediatric anesthetic. We show that acetyl L-carnitine (ALCAR) reverses ketamine-induced attenuation of heart rate and neurotoxicity (motor neurons, sensory neurons, serotonin and dopamine systems) in zebrafish embryos. However, it's not clear how ALCAR reverses ketamine-induced toxicities. In order to delineate the mechanism, we used 48 hours post fertilization (hpf) zebrafish embryos that were exposed to the drugs for 2 or 4h. In the 48 hpf embryos, 2 mM ketamine reduced heart rate in a 2- or 4-h exposure and 0.5 mM ALCAR neutralized this effect. ALCAR could reverse ketamine's effect, possibly through a compensatory mechanism involving extracellular Ca^{2+} entry through L-type Ca^{2+} channels that ALCAR is known to activate. Hence, we used verapamil to block the L-type Ca^{2+} channels. Verapamil was more potent in attenuating heart rate and inducing morphological defects in the embryos compared to ketamine at specific times of exposure. ALCAR reversed cardiotoxicity and developmental toxicity in the embryos exposed to verapamil or verapamil plus ketamine, even in the presence of 3,4,5-trimethoxybenzoic acid 8-(diethylamino)octyl ester (TMB-8), an inhibitor of intracellular Ca^{2+} release, suggesting that ALCAR acts via effectors downstream of Ca^{2+}. In fact, ALCAR's protective effect was blunted by oligomycin A, an inhibitor of ATP synthase that acts downstream of Ca^{2+} during ATP generation. We have identified, for the first time, a downstream effector of ALCAR that's critical in abrogating ketamine- induced developmental toxicities.

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Poster

598. Brain Wellness and Aging: Physiological and Molecular Correlates

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 598.01/N6

Topic: C.01. Brain Wellness and Aging

Title: Identification of putative neurogenesis-associated transcriptional biomarkers

Authors: *N. M. WALTON[1], S. MIYAKE[2], M. MATSUMOTO[2], H. ITO[1], K. TAJINDA[1]; [1]Neurosci., Astellas Res. Inst. of America LLC, Skokie, IL; [2]Neurosci. Res. Unit, Drug Discovery Res., Astellas Pharma Inc, Tsukuba, Japan

Abstract: Hippocampal neurogenesis is a fundamental constituent of learning and memory; alterations in this process have been implicated in a number of CNS diseases. However, despite the obvious contribution of this process to CNS function, dynamic descriptions of postnatal neurogenesis remain hampered by technical limitations. Current studies are largely constrained

to postmortem evaluation, preventing real-time evaluation of live populations. This is particularly true in human subjects, for whom consensus defined biomarkers do not exist.
In an effort to identify peripheral transcriptional biomarkers that mirror neurogenesis-specific transcriptional expression changes associated with hippocampal neurogenesis, we compared a number of microarray datasets from brain and peripheral blood mononuclear cells (PBMCs). Using reductive comparison, we identified transcripts that exhibited robust, conserved expression in both blood and brain. We then compared this dataset to hippocampal- and/or PBMC-derived samples isolated from rodent models of perturbed neurogenesis. These included voluntary exercise and chronic fluoxetine administration, as well as animal models that display alterations in early- and late stages of neurogenesis and/or neuronal maturation. To further refine gene expression changes to neural stem/progenitor cell-specific genes and/or populations, we compared hits identified in vivo to an in vitro neurogenesis assay containing an enriched population of primordial neuropoietic cells.
Using this approach, we were able to identify numerous examples of genes exhibiting concomitant transcriptional alteration in both hippocampal and PBMC samples. From this dataset, it may be possible to identify responsive elements in nucleated blood cells that retain a conserved transcriptional response to those of hippocampal stem/progenitor cells.

Disclosures: **N.M. Walton:** A. Employment/Salary (full or part-time): Astellas Pharma Inc. **S. Miyake:** A. Employment/Salary (full or part-time): Astellas Pharma Inc. **M. Matsumoto:** A. Employment/Salary (full or part-time): Astellas Pharma Inc. **H. Ito:** A. Employment/Salary (full or part-time): Astellas Pharma Inc. **K. Tajinda:** A. Employment/Salary (full or part-time): Astellas Pharma Inc.

Poster

598. Brain Wellness and Aging: Physiological and Molecular Correlates

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 598.02/N7

Topic: C.01. Brain Wellness and Aging

Support: NSF grant HRD-0802628

Title: Identification of withania somnifera active constituents on $GABA_{rho}$ receptors

Authors: ***H. AHMED**[1], A. LI[1], N. DARABEDIAN[2], F. ZHOU[1], A. RUSSO-NEUSTADT[1], A. LIMON[3];
[1]California State University, LA, Los Angeles, CA; [2]Univ. of Southern California, Los Angels, Los Angeles, CA; [3]Univ. of California, Irvine, Irvine, CA

Abstract: Recent scientific evidence suggests that hyperexcitability in neural circuits may be responsible for neurodegeneration and cognitive impairment during aging. This may, in turn, be due to an imbalance between excitation and inhibition neurotransmission or a reduction in GABAergic signaling. Thus, developing a way to prevent hyperexcitability in the brain can be an excellent strategy for preventing neuronal death and cognitive decline. *Withania somnifera* (WS), also known as ashwagandha, is herbal medicine that is been used for centuries in Ayurvedic medicine and is known as an adaptogenic plant because of its stress reducing capability. We have chosen to study this plant because evidence indicates that it has calming and GABA-enhancing capabilities. Our preliminary studies show that whole root WS modulates hippocampal activity and survival via a GABA-mimetic mechanism. In addition, the most widely studied active components of WS root extract, withaferin A and withanolide A, were not responsible for the observed GABA receptor activation. Thus, we hypothesize that other key WS constituents promote optimal hippocampal function and neuronal survival via GABAergic mechanism. To test this hypothesis, whole WS extracts have been separated into eight fractions using liquid-liquid extraction. To identify bioactive fractions with GABA activity, the *Xenopus laevis* oocyte model was utilized to study $GABA_{rho}$ ($GABA_A$ subtype) receptor function. Cloned $GABA_{rho}$ cDNA from rat brain was injected into *Xenopus* oocytes. Two days after injections, GABA currents were recorded from voltage clamped oocytes using microelectrodes filled with 3M KCl and membrane potential held constant at -80 mV. The oocytes were continuously perfused with ringer solution at room temperature, followed by perfusion of the eight extracted WS fractions at 1: 100 dilution to test their physiological activity. Results showed that two out of eight fractions (water and butanol) exhibit significant maximal GABA response as compared to 10 uM GABA as a control (One Way ANOVA, $p < 0.0001$). This finding supports our hypothesis that major WS constituents may be responsible for the GABA-mimetic activity and have specificity for $GABA_{rho}$ receptors as shown by significant GABA response in the two identified WS fractions and minimal response in the remaining six WS fractions. These results suggest that these constituents may be polar substances such as withanosides IV and VI, or small molecules such as amino acids. Understanding the mechanism of WS may be valuable for the development of pharmacological treatments for neurological disorders associated with GABAergic signaling dysfunction.

Disclosures: H. Ahmed: None. **A. Li:** None. **N. Darabedian:** None. **F. Zhou:** None. **A. Russo-Neustadt:** None. **A. Limon:** None.

Poster

598. Brain Wellness and Aging: Physiological and Molecular Correlates

Location: Halls B-H

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Program#/Poster#: 598.03/N8

Topic: H.03. Schizophrenia

Support: NIH Grant MH-058846

Emory SIRE Program

Title: Altered parvalbumin cell populations in dorsolateral prefrontal cortex after neonatal hippocampal damage in macaques.

Authors: *T. J. LIBECAP[1], A. J. HOWLEY[1], M. C. ALVARADO[3], J. BACHEVALIER[2,3], H. R. RODMAN[2];
[1]Neurosci. and Behavioral Biol., [2]Psychology, Emory Univ., Atlanta, GA; [3]Yerkes Natl. Primate Ctr., Atlanta, GA

Abstract: GABA, the primary inhibitory neurotransmitter in the mammalian brain, plays a fundamental role in the dorsolateral prefrontal cortex (dlPFC) under normal working conditions. In particular, the parvalbumin (PV) class of GABA interneurons is involved in sustaining the normal neuronal signaling that supports proper functionality of the dlPFC executive system. Because the dlPFC undergoes a protracted development, early disruption may play a role in working memory impairment and other cognitive deficits associated with neurodevelopmental disorders including schizophrenia (SCZ). Further, the hippocampus, a structure in the medial temporal lobe, matures relatively early in life and has been implicated in dlPFC development. Thus, we predicted that a neonatal hippocampal lesion would disrupt the normal PV-positive cell maturation within the dlPFC, consistent with an early limbic-prefrontal disconnection model of SCZ. In this study, we used tissue harvested from adult rhesus monkeys (*Macaca mulatta*) that had sustained either bilateral ibotenic acid lesions of the hippocampus (Neo-H, n = 4) or sham operations (Neo-C, n= 4) as neonates to analyze the relative density of PV-positive GABAergic interneurons in cortical layers IIIA and IIIB of Brodmann Area 46d.We used immunohistochemistry, Giemsa counterstaining, systematic random sampling, and the optical disector and optical fractionator stereological principles to visualize and quantify PV-immunoreactive neurons. We found a significantly higher density of PV-positive cells specifically within layer IIIA of the dlPFC of Neo-H monkeys relative to the Neo-C monkeys, both between the left hemispheres of the two groups and across the groups when both hemispheres were considered together. These results were confirmed by estimations of total PV-positive cell populations in layer IIIA of area 46d. Moreover, layer IIIA PV densities of the Neo-H group were significantly positively correlated with the extent of hippocampal damage. The finding of PV density changes in IIIA but not IIIB suggests that the alterations are related to specific components of GABAergic circuits rather than general disruption of PV expression. Furthermore, these results are consistent with functional connectivity changes and behavioral deficits observed in the same Neo-H cases. Ultimately, these findings have implications for how neonatal lesions of the hippocampus may affect vulnerable structures and disrupt cognitive processing, eventually leading to deficits characteristic of neurodevelopmental disorders including schizophrenia.

Disclosures: **T.J. Libecap:** None. **A.J. Howley:** None. **M.C. Alvarado:** None. **J. Bachevalier:** None. **H.R. Rodman:** None.

Poster

598. Brain Wellness and Aging: Physiological and Molecular Correlates

Location: Halls B-H

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Program#/Poster#: 598.04/N9

Topic: C.01. Brain Wellness and Aging

Support: ERC Starting Grant 282430

MRC Studentship

Title: Mitochondrial dynamics in parvalbumin interneurons

Authors: *G. KONTOU, N. F. HIGGS, G. LÓPEZ-DOMÉNECH, J. T. KITTLER; Neurosci. Physiol. and Pharmacol., Univ. Col. London, London, United Kingdom

Abstract: Parvalbumin interneurons (PV+INs) require very high amounts of energy to sustain their firing rates that are implicated in the generation of high frequency oscillatory behavior. It has been observed that these interneurons contain high levels of mitochondria which reflect the large energy utilization that takes place. Hence, they are susceptible to incidents of mitochondrial impairment as mitochondria provide the predominant source of energy. A shortage in energy provision, due to deficits in the ability of mitochondria to generate ATP and buffer calcium, may result in a dysfunction in PV+INs metabolic activity and signaling. These dysfunctions can cause aberrant rhythmic activities and cognitive decline which are observed in neurological and neuropsychiatric disorders. Neurons are characterized by an exaggerated polar morphology and therefore, specialized mechanisms have evolved to distribute mitochondria to sites of high energy demand in order to meet the metabolic needs of the cell. We have generated a conditional transgenic mouse line using the PV-Cre and the PhAM^{floxed} lines where mitochondria are fluorescently labeled with MitoDendra only in PV+INs. By employing organotypic brain culture preparations from young animals, we are able to investigate mitochondrial dynamics such as trafficking, fission and fusion using live two-photon and confocal microscopy. We show that the expression of MitoDendra under the parvalbumin promoter begins around P10 and is stabilized around P28 and that more than 90% of the Mitodendra-expressing cells are immunopositive for parvalbumin. This suggests that the PV-Cre MitoDendra mouse is a suitable model for the cell-type specific investigation of mitochondrial dynamics in PV+INs. Using live-imaging of *ex vivo* organotypic brain cultures, we have characterized the trafficking properties of mitochondria in PV+INs in the hippocampus under basal conditions. Additionally, MitoDendra can be successfully photoconverted for implementing single mitochondrion tracking experiments and for investigating fusion and fission. Here, we introduce for the first time a strategy to investigate mitochondrial dynamics in PV+INs in intact brain tissue. By taking advantage of this approach, we can now specifically manipulate mitochondrial trafficking and investigate how changes in

energy provision and calcium homeostasis could be implicated in the PV+INs physiological behavior and network activity.

Disclosures: G. Kontou: None. **N.F. Higgs:** None. **G. López-Doménech:** None. **J.T. Kittler:** None.

Poster

598. Brain Wellness and Aging: Physiological and Molecular Correlates

Location: Halls B-H

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Program#/Poster#: 598.05/N10

Topic: C.01. Brain Wellness and Aging

Support: 4R37AG004542-27

Gordon and Rose McAlpine Foundation for Neuroscience Research

Title: Aging-related increase in the Ca^{2+}-dependent slow afterhyperpolarization (sAHP) of neurons in layer III of the entorhinal cortex

Authors: *J. C. GANT, P. W. LANDFIELD;
Molec. and Biomed. Pharm, Univ. of Kentucky, Lexington, KY

Abstract: Normal aging results in a decline of cognitive function that has been associated with Ca^{2+} dysregulation in hippocampal CA1 pyramidal neurons of several species. Among the most consistent and well-studied electrophysiological markers of neuronal Ca^{2+} dysregulation is the age-related enlargement of the Ca^{2+}-dependent sAHP. This enlargement is generated by an increase in Ca^{2+} influx via L-type Ca^{2+} channels and enhanced Ca^{2+} release from ryanodine receptors. Most studies to date showing an aging-related increase in the sAHP have been conducted in hippocampal pyramidal neurons. Therefore, it is not clear whether this form of Ca^{2+} dysregulation is widespread across brain regions and cell types or is instead restricted primarily to the hippocampus. Resolving this question may substantially facilitate identification of the neuronal circuits involved in aging changes in memory processing. In particular, the extensive reciprocal connectivity between the Entorhinal cortex (EC) and hippocampus is well recognized to play an important role in memory formation but it is not known whether Ca^{2+} dysregulation extends to the EC. We obtained intracellular current clamp recordings from layer III neurons in entorhinal slices from young (3-5 months-old) and aged (20-22 months-old) male rats. Layer III of the EC contains a high proportion of pyramidal cells and essentially all neurons we recorded exhibited a sAHP. Results revealed a highly significant increase in the magnitude of the sAHP and sAHP duration in aged rat EC neurons compared to young rat EC neurons ($p<0.002$ and

p<0.0004 respectively). This aging-related increase was larger than is typically seen in CA1 neurons. Thus, at least one component of the EC projection to hippocampus exhibits strong evidence of Ca^{2+} dysregulation with aging that appears similar to the form found in CA1 pyramidal neurons.

Disclosures: J.C. Gant: None. **P.W. lANDFIELD:** None.

Poster

598. Brain Wellness and Aging: Physiological and Molecular Correlates

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 598.06/N11

Topic: C.01. Brain Wellness and Aging

Title: Effects of aging on the food intake and the function of the related synapse in the feeding behavior of *Aplysia* kurodai

Authors: *T. NAGAHAMA, R. ABE, M. MURAMATSU, A. KASHIMA;
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Abstract: In the wild animals of *Aplysia kurodai*, the birthdate of each individual cannot be determined. Therefore, we initially sought a reliable index of old age by taking the distinguished Japanese seasons into consideration. During and after the second half of May, we commonly find large amounts of animal eggs on the surface of rocks along the coast. And dead bodies of animals begin to appear, which increase in number with the passage of time. The body weights of the collected animals were found to decrease after May. We roughly classified animals collected before and after the second half of May as mature-CP and old-CP. Plots of internalized shell length (*S*) against body weights (*W*) gave distinct best-fit curves for mature-CP and old-CP. The *W/S* significantly decreased in the second half of June, suggesting that body weight decreases with age but the shell length is maintained in each animal. Therefore, collected animals were classified into mature and old animals by using the best-fit curves for mature-CP and old-CP. Whether the plots of the shell length against the body weight were located above the *W-S* curve for old-CP or below the *W-S* curve for mature-CP was used for our judgment on the old or mature animals, respectively. In the present experiments the effect of aging on the food intake was examined. When we measured amount of food intake every 2 hrs up to 8 hrs after providing food, the amount increased linearly in all animals but the rate was significantly lower in old animals than in mature animals. The amount of one-day food intake was also significantly lower in old animals. These results suggest that food intake may decline with age and this may cause weight loss in old animals. We next examined the effect of aging on the function of the related synapse in the feeding neural circuit. Then it was focused on the monosynaptic cholinergic

response in the jaw-closing (JC) motor neurons induced by the buccal multiaction (MA) neurons. We found that the inhibitory postsynaptic currents (IPSCs) significantly decreased with age. We previously showed that reduction of this synaptic response by the modulatory dopaminergic neuron (CBM1) may contribute to generation of the patterned jaw movements for rejection of food. Therefore, an injury of the cholinergic synaptic function with aging may partly contribute to the decline of the food intake.

Disclosures: **T. Nagahama:** None. **R. Abe:** None. **M. Muramatsu:** None. **A. Kashima:** None.

Poster

598. Brain Wellness and Aging: Physiological and Molecular Correlates

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 598.07/N12

Topic: C.01. Brain Wellness and Aging

Support: DP1 AG047744-03 Pioneer Award

Title: Dysregulation of mitochondrial morphology during aging in the monkey dorsolateral prefrontal cortex

Authors: *D. DATTA, Y. M. MOROZOV, C. D. PASPALAS, A. F. T. ARNSTEN;
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Abstract: The dorsolateral prefrontal cortex (DLPFC) is one of the most newly evolved regions in the primate brain and mediates highest-order cognitive functions. A prototypic cognitive function executed by the DLPFC involves working memory (WM), the ability to use representational knowledge to regulate behavior, thought and emotion. Within DLPFC, modules of pyramidal cells in layer 3 microcircuits engage in recurrent excitation to sustain persistent neural firing required to maintain WM, so-called “Delay cells”. These DLPFC layer 3 microcircuits are highly vulnerable to age-related cognitive decline and to genetic insults in psychiatric diseases such as schizophrenia. For example, the firing of “Delay cells” and dendritic spine density within DLPFC layer 3 microcircuits decrease with advancing age. Since the recurrent excitation required to sustain WM is highly energy demanding, the PFC contains a higher density of mitochondria compared to other cortical regions. However, whether dysregulation of mitochondria contribute to the neurobiological underpinnings of age-related cognitive decline is unknown. Here, we tested the hypothesis that mitochondrial morphology in DLPFC is altered with aging and that impairments in mitochondrial dynamics would be most pronounced in DLPFC layer 3. Using stereological electron microscopy with ultrathin serial sections, we performed 3D reconstructions and volume measurements of mitochondria from

young adult rhesus macaques (7-11 y) and aged rhesus macaques (26, 27, 31 and 33 y). Qualitatively, aged mitochondria exhibited frequent swellings next to highly constricted areas, consistent with "incomplete" mitochondrial fission. Quantitatively, the percentage of mitochondrial profiles (n=365) with "incomplete" fission was ~0% in young adults; however, the frequency of "incomplete" fission profiles (n=774) increased in a dramatic fashion across all laminar locations during aging (43%, 26y; 10%, 27y; 40%, 31y; 18%, 33y). Although individual variability in monkeys was present, layer 3 showed a progressive, linear increase in dysregulated mitochondrial dynamics. These "incomplete" fission mitochondria in aged DLPFC are also associated with cisterns of ER tubules suggesting that aberrant ER-mitochondria processes might induce impairments in mitochondrial dynamics. These findings reveal a robust pathological abnormality that may contribute significantly to age-related cognitive decline in the DLPFC. Future studies will address how intracellular signaling pathways that modulate the physiological strength of recurrent DLPFC network connections can synergistically produce these pathological hallmarks.

Disclosures: D. Datta: None. **Y.M. Morozov:** None. **C.D. Paspalas:** None. **A.F.T. Arnsten:** None.

Poster

598. Brain Wellness and Aging: Physiological and Molecular Correlates

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Topic: C.01. Brain Wellness and Aging

Support: Pilot COBRE on Aging and Regenerative Medicine A50886G5 5P20GM103629-03 to RM

Startup Funds from the Tulane University School of Medicine to RM

Title: Hyperpolarized resting membrane potential of fast spiking neurons: a putative cause of decreased activity-dependent IPSCs in pyramidal neurons of the barrel cortex of aged mice

Authors: *I. R. POPESCU[1], K. Q. LE[2], R. MOSTANY[1];
[1]Pharmacol., [2]Neurosci. Program, Tulane Univ., New Orleans, LA

Abstract: Previous studies using long-term *in vivo* two-photon imaging of the dendritic spines of layer 5 pyramidal neurons in the primary somatosensory cortex have shown that advanced age brings about an increase in the spine turnover rate, potentially interfering with memory storage. Excitatory and inhibitory synaptic inputs to pyramidal neurons may modulate dendritic spine

dynamics either directly by controlling the resting membrane potential and Ca^{2+} influx or by affecting the expression of proteins which play a role in the structure of dendritic spines. Identifying which synaptic inputs are altered by age and what underlies these changes will provide specific targets for interventions aimed at redressing the acceleration of spine turnover. The pyramidal (excitatory) neurons in the somatosensory barrel cortex receive GABAergic inputs from several types of interneurons, including parvalbumin-expressing fast spiking (FS) neurons, somatostatin-expressing neurons and vasoactive intestinal peptide-expressing neurons. Using whole-cell patch clamp recordings in brain slices, we are pinpointing age-related changes in this circuit. Our recordings from pyramidal neurons indicate a decrease in sIPSC frequency and amplitude, as well as a lack of TTX suppression of IPSCs in layer 5 pyramidal neurons from aged mice (> 18 mo), compared to pyramidal neurons from young adult mice (3-6 mo). We hypothesized that a likely cause of this age-related decrease in IPSCs in pyramidal neurons is a decline in the activity of FS neurons, since they provide the majority of perisomatic inputs to pyramidal neurons. To ascertain this, we recorded from FS neurons classified as putative basket or chandelier cells based on their electrophysiological properties, and found an increase in the resting potential of basket cells in aged mice. The age-related hyperpolarization of the resting potential of basket cells is expected to decrease action potential frequency and may also decrease the probability of release of GABA, thereby contributing to the observed deficit of inhibition in pyramidal neurons. Our results suggest that deficits in inhibitory synaptic inputs to pyramidal neurons could be playing a role in the synaptic instability observed in the cortical circuits of aged mice, thus making fast spiking basket cells a potential target for the development of therapeutic interventions designed to delay the onset of aging-related brain decline and hence prolong the quality of life and welfare of the elderly.

Disclosures: I.R. Popescu: None. **K.Q. Le:** None. **R. Mostany:** None.

Poster

598. Brain Wellness and Aging: Physiological and Molecular Correlates

Location: Halls B-H

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Program#/Poster#: 598.09/N14

Topic: C.01. Brain Wellness and Aging

Title: Proteomic analysis of human plasma as a source for diagnostic and therapeutic targets for aging-related disorders

Authors: B. SZOKE[1], W. KIM[1], T. WILLIS[2], *S. P. BRAITHWAITE[1];
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Abstract: Blood forms a systemic milieu that connects all tissues in higher order animals. As such, it reflects the state of the cells, organs, and the entire organism, and has extensively been used as a source of diagnostic biomarkers. Accumulating evidence from parabiosis experiments suggests that it can also influence function, with young blood being beneficial and old blood detrimental, particularly for cognitive performance. Similar effects have been observed for plasma suggesting that these functional effects are mediated by soluble factors rather than cells. To identify factors that can potentially serve as targets of novel diagnostics and therapeutics for age-associated pathologies, we set out to understand aging-related changes in the human plasma proteome.

Proteomic analysis of plasma is highly challenging due to the very large number of proteins and proteoforms present in plasma (10^4 - 10^5) combined with their wide dynamic range in abundance (up to 10^{10}-fold). We employed complementary approaches, both affinity based targeted and mass spectrometry based discovery proteomics analyses. Using high quality human plasma samples from Grifols at 18, 30, 45, 55, and 66 years of age, we analyzed >1000 proteins and detected significant age-dependent changes in over 100 proteins. Approximately half of the changing proteins increased and half decreased from 18 to 66 years of age. Analysis over the multiple age groups provided high resolution and revealed differential time courses of changes of individual proteins. The dynamics of protein levels over aging adds a valuable new dimension to understanding the role of age-dependent plasma proteins. These results provide a comprehensive basis for identifying biomarkers of biological age and suggest the existence of both pro-youth and pro-aging factors in human plasma. The understanding of the changes in the plasma proteome can also inform selection of potential targets for therapies for age-associated diseases like Alzheimer's disease.

Disclosures: **B. Szoke:** A. Employment/Salary (full or part-time): Alkahest. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alkahest. **W. Kim:** A. Employment/Salary (full or part-time): Alkahest. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alkahest. **T. Willis:** A. Employment/Salary (full or part-time): Grifols. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Grifols. **S.P. Braithwaite:** A. Employment/Salary (full or part-time): Alkahest. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alkahest.

Poster

598. Brain Wellness and Aging: Physiological and Molecular Correlates

Location: Halls B-H

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Topic: C.01. Brain Wellness and Aging

Support: NIH Grant NS070825

The Aging Institute of the University of Pittsburgh and University of Pittsburgh Medical Center

Title: Environmental isolation impairs measures of brain health

Authors: *S. CASTRO-SCHEIRER[1], J. D. JAUMOTTE[1], R. J. SMEYNE[2], M. J. ZIGMOND[1];
[1]Neurol., Univ. of Pittsburgh, Pittsburgh, PA; [2]Developmental Neurobio., St. Jude Children's Res. Hosp., Memphis, TN

Abstract: Many individuals experience an impoverished lifestyle often associated with cognitive, emotional, and motor decline and can lead to a reduced life span. Such individuals include elderly living with little social contact, the homeless, and those living in jails and prisons, particularly those in solitary confinement. We are assessing the impact of housing under isolation conditions using a behavioral test battery and biochemical, molecular biological, and anatomical methods. F344/BN male rats 18 month old at the outset of our study were housed either individually in a standard shoebox cage (18 cm W x 38 cm D x 27 cm H; SE) or in groups of 6 in a relatively enriched environment (1 m W x 1m D x 0.6m H; EE) containing running wheels, tunnels, platforms, and toys. Body weights remained relatively stable in the SE rats but increased by an average of 10% for the EE rats over a 4-month period. The SE animals showed relatively little behavioral activity, which was consistent with the small space in which they were housed. In contrast, the EE rats showed a good deal of exploration, climbing, playing, and social interaction. After 4 months, all rats were euthanized, brain, peripheral tissues, and blood collected, and the brain dissected into several regions. Assays are being performed and and comparison made between SE and EE groups. Although not all the differences were statistically significant, a number of promising trends have already been observed. For example, we found that the SE rats housed in isolated, impoverished conditions had a 72% decrease in BDNF. These rats also had a 37% decrease in the ratio of dopamine (DA) metabolites to DA and a 30% decrease in the level of phosphorylated tyrosine hydroxylase in the striatum; both of which suggested a decrease in DA synthesis and release in that structure. There was a five-fold increase in mitochondrial DNA damage levels in hippocampus in the SE rats. In addition, substantia nigra from rats in the SE showed a number of significant differences in mRNA expression, including changes in Azin1, Tssc4, Ddit4, Nfkb1a, Pdk4, and Sgk1 (downregulated) and Cxcl13 and

slc47a1 (upregulated). Additional assays are ongoing. Thus far our results indicate that isolated housing produces significant changes consistent with decreased neuroplasticity. In a parallel study, a marked change in cytokine levels in response to an LPS challenge was observed as a result of isolated housing (see Jaumotte et al., at this meeting). These results suggest that isolated, impoverished living conditions can produce profound changes in the brain that may be at least partially responsible for the behavioral impairments observed in people experiencing such conditions.

Disclosures: S. Castro-Scheirer: None. **J.D. Jaumotte:** None. **R.J. Smeyne:** None. **M.J. Zigmond:** None.

Poster

598. Brain Wellness and Aging: Physiological and Molecular Correlates

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Topic: C.01. Brain Wellness and Aging

Support: NIH Grant NS070825

ALSAC

The Aging Institute of University of Pittsburgh and University of Pittsburgh Medical Center

Title: Isolated housing decreases the immune response in sera and brain following exposure to a bacterial toxin in older rats

Authors: *J. D. JAUMOTTE[1], S. L. CASTRO[1], M. J. ZIGMOND[1], R. J. SMEYNE[2]; [1]Neurol., Univ. of Pittsburgh, Pittsburgh, PA; [2]Developmental Neurobio., St. Jude Children's Res. Hosp., Mephis, TN

Abstract: As people age they often are more likely to become ill. Among the many factors that could explain their reduced health span is the increasing isolation commonly experienced by the elderly, as well as other segments of our society. This, in turn, can be associated with an impaired immune system, including a decline in B- and T-cell production, which is reflected by changes in the expression of cytokines produced in response to exposure of viral or bacterial agents. In this experiment, we examined the immune response elicited by exposure to lipopolysaccharide (LPS), an endotoxin produced by bacteria. We first examined a small dose-response curve for LPS (0-2 mg/kg, i.p.) in 28-month-old male Fisher 344/Brown Norway hybrid rats (F344/BN) to determine the highest tolerable dose of a single intraperitoneal injection of

LPS. We then used an intermediate dose from that analysis (empirically determined to be 1 mg/kg) and delivered it to male F344/BN rats housed housed in our facility for 8 months beginning at 19 months of age. The two groups studied were either singly housed in a standard shoebox cage (18 cm W x 38 cm D x 27 cm H; SE) or a relatively enriched environment consisting of a large cage (1 m W x 1m D x 0.6m H) containing 6 rats, running wheels, tunnels, platforms, and toys (EE). Seven days after the LPS injection all animals were euthanized and brain and serum collected. Using a Luminex multiplex assay kit, we observed that several cytokines and chemokines were significantly altered in both sera and brain from isolated animals in comparison to those in enriched housing. The pattern of change indicated that in isolation led to a reduction in the immune response. Cytokines and chemokines that changed in response to isolation included G-CSF (sera), IL-1 alpha (sera and brain), IL-1-beta (sera and brain), IL-4 (sera and brain), IL-6 (sera), IL-10 (sera), IP-10 (sera), INF-gamma (sera and brain). These data suggest that older, isolated animals have a less reactive immune response than their more enriched counterparts, which could indicate a lowered ability to fight off infections or stave off neurological disease that have an immunological component, including Alzheimer's and Parkinson's disease.

Disclosures: J.D. Jaumotte: None. **S.L. Castro:** None. **M.J. Zigmond:** None. **R.J. Smeyne:** None.

Poster

598. Brain Wellness and Aging: Physiological and Molecular Correlates

Location: Halls B-H

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Program#/Poster#: 598.12/N17

Topic: C.01. Brain Wellness and Aging

Support: NRF-2015R1D1A3A01020635

Title: Age-related changes of PGRN expression in the gerbil hippocampus

Authors: K. LEE[1], D. YOO[3], J. CHUNG[2], I. HWANG[3], *J. CHOI[4];
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Abstract: Progranulin (PGRN) is a growth modulating factor which is known to be related with normal brain development and neurodegenerative disorders. In the present study, we compared immunoreactivity and distribution of PGRN between the adult and aged gerbil hippocampus.Immunoreactivity of PGRN was detected in the all hippocampal sub-regions and

pyramidal cells in the CA 2/3 region were more darkly stained than those in the CA1 in the both groups. However, PGRN immunoreactivity in the aged hippocampus was significantly decreased compared to that in the adult group. Double immunofluorescence showed that PGRN-immunoreactive structures were detected in cytoplasm of NeuN-positive neurons and Iba-1-positive microglia in the hippocampus of the both groups, but, in the GFAP-positive astrocytes, PGRN-positive structures were observed around and in their processes.In conclusion, PGRN immunoreaction was detected throughout the all hippocampal sub-regions in both adult and aged gerbil. However, marked decrease of PGRN immunoreactivity in the hippocampus was observed in the aged gerbil. Therefore, the decrease of PGRN immunoreactivity in the hippocampal neurons in aged hippocampus may be associated with age-related changes of hippocampal function.

Disclosures: K. Lee: None. **D. Yoo:** None. **J. Chung:** None. **I. Hwang:** None. **J. Choi:** None.

Poster

598. Brain Wellness and Aging: Physiological and Molecular Correlates

Location: Halls B-H

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Topic: C.01. Brain Wellness and Aging

Support: American Heart Association (AHA) 44081 (M.J.P)

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School of Dentistry URC pilot project (A.N.A.)

Title: Peripheral and spinal postoperative anti-hypersensitivity by mu-opioids DAMGO and buprenorphine in aged versus adult mice

Authors: *J. M. MECKLENBURG[1], M. J. PATIL[1], W. KOEK[2], A. N. AKOPIAN[1];
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Abstract: Background: Postoperative pain management in the elderly is challenging. This challenge arises due to the changes in their anatomy, physiology, biochemistry and especially wound healing dynamics, which are negatively affected by aging (Benyamin et al., 2008; Portenoy et al., 2004). In addition, old age could affect plasticity in the nociceptive pathway. These changes in the elderly may alter opioid effects, the main medicine for pain management. Therefore, we have investigated whether old age alters opioid-induced postoperative anti-hypersensitivity. **Methods:** Postoperative conditions were modeled by plantar incision in mice (Pogatzki and Raja, 2003). To evaluate the effects of a mu-opioid (DAMGO) and a partial opioid

antagonists/agonist (buprenorphine) on postoperative anti-hypersensitivity in adult and aged mice, thermal and mechanical nociception was measured using Hargreaves apparatus and Dynamic Plantar Aesthesiometer, respectively. Various doses of each drug were administered via local (surgical site) or intrathecal (spinal) injections and pain measurements were recorded 30 minutes and 120 minutes post opioid injection. **Results:** Locally injected DAMGO and buprenorphine had a mild effect on postoperative anti-hypersensitivity in adults and aged mice. However, this effect was not peripherally mediated. In contrast, spinal injection of DAMGO and buprenorphine showed very robust anti-hypersensitivity in adults and to lesser extend in aged mice. Importantly, peripheral and especially spinal DAMGO and buprenorphine-induced postoperative anti-hypersensitivity was substantially more effective in adult compared to aged mice. Thus, dose-response curves were leftward shifted in adults compared to aged mice. The dose-response curve for analgesic effects of buprenorphine was bell-shaped. Finally, buprenorphine had a stimulatory side effect in adults, but not in aged mice, that significantly affected the locomotor activity of adult mice. **Conclusion:** Our data indicates that mu-opioids are less effective for postoperative anti-hypersensitivity in aged as it is in adult mice. Nevertheless, the bell-shaped dose-response curve for buprenorphine analgesic effects and absence of stimulatory side effects at analgesic dosages in aged mice could make buprenorphine a better candidate for postoperative pain management in the elderly.

Disclosures: J.M. Mecklenburg: None. **M.J. Patil:** None. **W. Koek:** None. **A.N. Akopian:** None.

Poster

598. Brain Wellness and Aging: Physiological and Molecular Correlates

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Topic: C.01. Brain Wellness and Aging

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Title: Genetics of co-regulation of iron, copper, and zinc in mouse brain

Authors: P. JIMENEZ[1], W. ZHAO[2], L. LU[2], *B. C. JONES[2];
[1]Univ. of Memphis, Memphis, TN; [2]Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Iron, copper and zinc are important trace nutrients for development and functioning of the brain. All three require tight homeostatic regulation and there is evidence for co-regulation in various brain regions. Deficiency in each of the metals may have developmental or other functional consequences and excess iron and copper, especially, is implicated in neurodegeneration. In our work, we are interested in the co-regulation among these metals both within and across brain regions.
Twenty strains of the BXD panel of recombinant inbred mice and their parental strains (C57BL/6J and DBA/2J) were analyzed for hippocampal, ventral midbrain and cortex Fe, Cu and Zn content. The age of the mice was 60-120 days of age.
The analysis of Fe, Cu, Zn was performed by X-ray fluorescence spectroscopy (TXRF) using a S2 PICOFOX Spectrometer for Trace Analysis (Bruker, AXS Inc., Madison, WI). This gave us the Fe, Cu and Zn values which were then normalized to tissue weights (µg/g). Correlational analyses including genome mapping were performed using Genenetwork, a public database (http://www.genenetwork.org). The results showed that in the ventral midbrain, iron and copper and copper and zinc were significantly correlated. In the hippocampus, copper and zinc were significantly correlated and in the cortex, none of the metals showed significant associations; however latent associations were revealed by principal components analysis of the strain means. The data thus show that co-regulation of these trace metals are highly tissue-specific. Moreover, we were able to map one quantitative trait locus for the three metals in the ventral midbrain as a principal component, on chromosome 12, near the *Ighg* gene. This gene codes for immunoglobulin heavy chain (gamma polypeptide). It is *cis*-regulated and its expression correlates 0.83 with the principal component for the three metals in the ventral midbrain, thus making it a candidate gene. We performed similar analyses for hippocampus and cortex and while we found suggestive QTL for each, we have yet to identify candidate genes. This will require the analysis of more of the BXD strains.
This work is the first of its kind to reveal interrelationships among Fe, Cu and Zn in brain, using a systems genetics/systems biology approach.

Disclosures: **P. Jimenez:** None. **W. Zhao:** None. **L. Lu:** None. **B.C. Jones:** None.

Poster

598. Brain Wellness and Aging: Physiological and Molecular Correlates

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Program#/Poster#: 598.15/O2

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant DK007778

NIH Grant AG037868

Title: Gaboxadol as a drug intervention for psychosocial stress in young and aged male F344 rats

Authors: *E. M. BLALOCK[1], S. QUTUBUDDIN[2], K. STAGGS[2];
[1]Dept Pharmacol, [2]Pharmacol. and Nutritional Sci., Univ. Kentucky Coll Med., Lexington, KY

Abstract: Psychosocial stress (PS) occurs when a non-noxious stimulus (e.g., loss of a loved one, solitary confinement) provokes a physiological stress response. PS can have negative systemic effects (e.g., elevated immune signaling, sleep loss, cognitive deficits) that are similar to those observed in aging. Further, new onset PS becomes more prevalent with aging. Despite this, relatively few studies have investigated the relationship between aging and PS. In prior work, we demonstrated that aged animals were hyporesponsive to PS, and that both young stressed animals and aged animals in general had reduced deep sleep. Because deep sleep loss exacerbates immune signaling, worsens cognition, and is lost with age, here we tested a pharmacologic deep-sleep promoting intervention on PS in young and aged animals. We hypothesized that the deep sleep promoting drug, gaboxadol (GBX), would improve PS outcomes in young animals and would generally improve age-related cognitive and sleep activity measures. To test this, young (3 mos) and aged (19 mos) male Fischer 344 rats were divided into four groups: control vehicle, control GBX, stress vehicle, stress GBX. Restraint (3h/ day, 4 days) was used as a model of PS. All animals were trained in the Morris water maze during these four days and movement activity was measured in the animals' home cages throughout. In line with previous work, young animals suffered cognitive deficits. Consistent with our hypothesis, PS-induced deficits in young animal probe trial performance were rescued by GBX, and GBX reduced home cage activity in stressed but not control young subjects. Further, as in prior studies, aged animals were hyporesponsive to PS, and did not respond to GBX. This work suggests that deep-sleep promoting therapies can be effective for combating PS-induced cognitive deficits in young, but not aged animals, and further highlight the need to evaluate interventions in age-appropriate models.

Disclosures: E.M. Blalock: None. **S. Qutubuddin:** None. **K. Staggs:** None.

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 599.01/O3

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: African green monkeys as a model for Alzheimer Disease: RNA transcriptomics and CSF biomarker evaluation

Authors: *P. E. CRAMER, K. TANIS, Y. WANG, K. LODGE, I. HAYASHI, C. ZERBINATTI, J. RENGER;
Merck Sharpe & Dohme, West Point, PA

Abstract: While many preclinical models of Alzheimer disease (AD) are reported, none fully recapitulates the features of human disease. We sought to determine whether higher order species, specifically non-human primates, the vervet African Green Monkey (AGM) or the rhesus macacque, could prove to be a more valid and translational model of AD. We analyzed the gene expression profiles of the dorsolateral prefrontal cortex and the visual cortex from a cohort of AGMs ranging in age from 6-33 years and a cohort of rhesus ranging from 3-33 years. We report gene expression changes in aged AGM cortex that were previously published to occur selectively in affected brain areas of human AD. These AD-specific changes were not observed in the aged rhesus. Additionally, we found changes in inflammatory cytokines and microglial genes in both AGM and Rhesus cortex that are consistent with the changes in the aging human brain that are accentuated in AD (Podtelezhinikov, A. et al, PLOS One 2011). We further evaluated cerebrospinal fluid (CSF) from the AGMs and found increases in total tau, phospho-tau, and decreases in A-beta42 levels in aged compared to young AGMs, also consistent with human AD biomarker changes. These data along with our additional characterization of cholinergic-related behavioral deficits in aged AGMs and AD-specific histopathology shown in Vardigan et al. and Gentzel et al., respectively, further suggest that the AGMs represent a novel and potentially useful translational model of AD.

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Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 599.02/O4

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: African green monkeys as a model for Alzheimer's Disease: evidence from cognitive measures and response to pharmacology.

Authors: *J. M. USLANER, J. VARDIGAN, V. GAKHAR;
Merck & Co. Inc, West Point, PA

Abstract: There are several key characteristics of Alzheimer's Disease (AD), perhaps most notably loss of cholinergic neurons, A-Beta aggregates (plaques), tau aggregates (tangles), cognitive dysfunction, and old age. Of particular value would be a preclinical model that exhibits all of these characteristics. Whereas rodent models exist in which many of these features are present, the uncertainty of translating pharmacology from rodent to human limits the value of these rodent models. Developing a non-human primate model of AD with validity to the human disease could dramatically improve the ability to discover novel therapeutics. The African Green monkey (AGM) may offer a special opportunity toward this goal. AGM have been shown to develop age-dependent A-Beta pathology, and we have recently shown that other biomarkers associated with AD are present in these animals (see posters from Cramer et al. and Gentzel et al). We therefore hypothesized that AGMs would show an age-dependent deficit on cognition tasks and that symptomatic treatments which improve cognition might be efficacious in improving cognitive performance in this animal model. To address this hypothesis, we trained young and aged AGMs in the Object Retrieval Detour (ORD) task, which is believed to be dependent on prefrontal cortex function and is sensitive to cholinergic disruption. We report that under vehicle conditions, aged AGM perform significantly poorer than younger monkeys on ORD. We also report that drugs which enhance cholinergic function were able to improve this deficit in aged AGM. Specifically, the acetylcholinesterase inhibitor donepezil (0.25 and 0.5mg/kg, IM) and the M1 muscarinic positive allosteric modulator PQCA (1mg/kg IM) produced a significant improvement in performance in aged AGM. These results, along with our additional characterization of CSF biomarkers, RNA sequencing, and histology shown in Cramer et al and Gentzel et al. suggest that the AGMs represent a novel and potentially very important model of mild/moderate AD.

Disclosures: **J.M. Uslaner:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); All authors are employees at Merck and may own stock in Merck & Co. **J. Vardigan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); All authors are employees at Merck and may own stock in Merck & Co. **V. Gakhar:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); All authors are employees at Merck and may own stock in Merck & Co..

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 599.03/O5

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: African Green Monkeys as a Model for Alzheimer's Disease: Effects of aging on brain histopathology

Authors: *R. C. GENTZEL, B. CONNOLLY, P. MANFRE, J. J. RENGER, P. CRAMER, J. USLANER;
Merck & Co., West Point, PA

Abstract: Pathological hallmarks of Alzheimer's disease (AD) include amyloid beta (A-Beta) and tau deposits in the brain, known respectively as plaques and tangles. Identification of animal models which recapitulate this pathology and the temporal pattern in which it occurs would improve the likelihood of identifying a treatment for AD. Non-human primates are attractive candidates for such a model due to the ease of translating research findings from higher-order species to humans. Given that the African Green Monkey (AGM) has been reported to have an age-related increase in A-Beta pathology, here we further characterized the histopathology these animals develop with age and compare it to the documented pathology of AD. We acquired a cohort of AGM brains, ranging from young to elderly adult, and performed immunohistochemistry on coronal sections through the frontal cortex, hippocampus, and occipital cortex. We confirmed that plaques increase in the cortex as a function of age. Tangles, which typically occur after the formation of plaques in humans with AD, were noted in the hippocampus from two old monkeys. Markers of inflammation and gliosis were associated with the presence of plaques, and further reflective of AD pathology. Additional work by Cramer et al. (see poster) has demonstrated that changes in AGM CSF biomarkers and gene expression are similar to that observed in AD patients. Furthermore, Vardigan et al. (see poster) has demonstrated that AGMs suffer cognitive decline with age, which can be alleviated with AD therapeutics. In totality, these data suggest that AGMs may serve as a novel model of mild-to-moderate AD.

Disclosures: R.C. Gentzel: A. Employment/Salary (full or part-time): Merck & Co. **B. Connolly:** A. Employment/Salary (full or part-time): Merck & Co. **P. Manfre:** A. Employment/Salary (full or part-time): Merck & Co. **J.J. Renger:** A. Employment/Salary (full or part-time): Merck & Co. **P. Cramer:** A. Employment/Salary (full or part-time): Merck & Co. **J. Uslaner:** A. Employment/Salary (full or part-time): Merck & Co..

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 599.04/O6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R21091969

Title: Pathological characterization of a novel transgenic rat model of cerebral amyloid angiopathy with microhemorrhage

Authors: *F. XU;
Neurolog. Surgery, Stony Brook Univ., Stony Brook, NY

Abstract: Cerebral amyloid angiopathy (CAA) is a prevalent cerebral small vessel disease in the elderly that causes cerebral hemorrhage, is a common co-morbidity of Alzheimer's disease (AD) and contributes to vascular cognitive impairment and dementia (VCID). Additionally, familial forms of CAA occur due to specific mutations within the amyloid β-protein (Aβ) including Dutch E22Q and Iowa D23N. Although much work has been performed previously to study CAA using various human AβPP transgenic mice these investigations have been somewhat limited with this species. On the other hand, rats provide a more advantageous species to model human cerebral disease due to their closer link to humans, larger brain size, higher white matter volume and more sophisticated cognitive testing. Here, we present the generation and pathological characterization of a novel transgenic rat to model human CAA. The rats, designated rTg-SwDI, were designed to express human AβPP and produce familial Dutch/Iowa CAA mutant Aβ in brain. rTg-SwDI rats develop progressive and extensive small vessel CAA starting at about 3 months of age in the cortex, thalamus and hippocampus. As rTg-SwDI rats age to 12 months they develop many of the pathological features of human CAA including dilated perivascular spaces, microinfarcts and numerous microbleeds that can be detected by both histological staining and MR imaging. Progressive small vessel disease in rTg-SwDI rats is accompanied by deteriorated cognitive performance. Our findings indicate that the novel rTg-SwDI rats provide an exciting and improved model for studying CAA and its contribution to VCID.

Disclosures: F. Xu: None.

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 599.05/O7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR Grant

CCNA Grant

Western University

Title: Assessing cognitive dysfunction, neuroinflammation and degeneration following an endothelin-1 induced stroke injury in an APP transgenic rat

Authors: *A. M. REGIS[1], L. WANG[1], B. ALLMAN[1], V. HACHINSKI[2], S. N. WHITEHEAD[1]; [1]Anat. & Cell Biol., [2]Clin. Neurolog. Sci., Western Univ., London, ON, Canada

Abstract: It is estimated that by age 60, one in three individuals will develop Alzheimer's disease (AD), suffer a stroke, or experience both. While traditionally regarded and treated as distinct conditions, the roles of Alzheimer's disease (AD) and stroke as interacting ailments has attracted interest, though a conclusive pathophysiological link has yet to be determined.
Using an *in vivo* rat model, it is hypothesized that following ischemic stroke, a transgenic rat model carrying human amyloid precursor protein (APP) mutations will display enhanced stroke-related pathology, heightened neuroinflammation, and more severe cognitive decline.
To model AD pathology, our study uses a genetically engineered rat model exhibiting mutations in APP; this model overproduces the human pathogenic protein attributable in AD called amyloid-β. To model an ischemic stroke injury, a unilateral striatal injection of endothelin-1, a potent vasoconstrictor, is utilized.
The proposed models are studied both in combination as well as singularly over a period of four months post-Endothelin-1 injury. A variety of behavioural tests will be used to determine any apparent cognitive and motor deficits, including Attentional Set-Shifting, Novel Object Recognition, Exploratory behavior analyses, as well as analyses of gait and limb co-ordination. Histological analytical methods will be used to determine any mechanistic changes at the cellular level in our injured animals. These strategies will help us better understand how stroke and AD manifest themselves in combination throughout the disease processes.
Preliminary results from our laboratory using rat models suggest that in the presence of amyloid deposition, facets of stroke including infarct size and the degree of neuroinflammation are heightened. These pathological effects are shown to be translated into behavioural deterioration in rodent models of AD and stroke. However, further studies are required to truly understand the interactions between amyloid toxicity, ischemic stroke damage, and neuroinflammation.
Upon defining the specific pathological mechanisms, use of therapeutic intervention may be employed. Overall, with a better understanding of the mechanistic roles of stoke and AD pathology on resultant cognitive impairment, it may be possible to employ strategies in rodents to minimize post-stroke cognitive burden; interventions that may become a translational strategy in humans who are susceptible to stroke and Alzheimer's disease related cognitive impairment.

Disclosures: A.M. Regis: None. **L. Wang:** None. **B. Allman:** None. **V. Hachinski:** None. **S.N. Whitehead:** None.

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

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Program#/Poster#: 599.06/O8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Supported by the National Research Council of Science & Technology (NST) grant by the Korean government (MSIP) (No. G15120 and CRC-15-04-KIST)

Title: Impairments of working memory in 5XFAD mice

Authors: *J.-S. HAN[1], H.-A. KIM[1], W. JEON[2,3];
[1]Konkuk Univ., Seoul, Korea, Republic of; [2]Korea Inst. of Oriental Med., Daejeon, Korea, Republic of; [3]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder. Early diagnosis and treatment of AD is critical for delaying its progression, but early detection of AD has not been successful. In order to provide a research platform of animal model for early detection of AD, the present study was conducted to examine cognitive impairments and neuropathological characteristics of 4 months old 5X familial AD (5XFAD) transgenic mice, because, at 6 months of age, cognitive impairments and neuropathological characteristics are clearly observed in 5XFAD mice. Capacity of working memory was measured in 5XFAD transgenic mice, using novel object recognition task. Novel object recognition task was performed at various intervals (10 min, 1 h, 4 h, and 24 h) to measure retention of recognition memory. Because early or mild AD patients show impairments in delayed recall, it is expected that 5XFAD mice would show worse performances at the intermediate retention intervals than non-Tg control mice. Consistent with our expectation, the investigation ratio of 5XFAD mice was significantly lower than those of non-Tg control mice at 4 h interval (Mann-Whitney U test, $p < 0.05$). The results indicate that the capacity of working memory was impaired in 5XFAD mice. In addition, accumulation of amyloid β 42 was measured in the brain structures responsible for working memory, using the immunohistochemistry. These findings indicate that cognitive impairments and neuropathological characteristics of 4 months old 5XFAD mice would provide a research platform for studying early diagnosis and treatment of AD. Supported by the National Research Council of Science & Technology (NST) grant by the Korean government (MSIP) (No. G15120 and CRC-15-04-KIST).

Disclosures: **J. Han:** None. **H. Kim:** None. **W. Jeon:** None.

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

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Program#/Poster#: 599.07/O9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Velux Stiftung

the Fonds de recherche du Québec-Santé (FRQS)

Title: Retinal function and structure analyses in transgenic APP_{swe}/PS1ΔE9 mice, a model for Alzheimer's disease

Authors: *S. M. JOLY, S. LAMOUREUX, V. PERNET;
Ophthalmology, Ctr. De Recherche Chuq/Université Laval, Quebec, QC, Canada

Abstract: In this study, the structure and function of the retina were studied in APP_{swe}/PS1ΔE9 transgenic mice, a well-accepted experimental model for Alzheimer's disease (AD). Ocular manifestations of the disease in this model are controversial. Previous studies reported amyloid beta (Aβ) plaque formation in the retina and visual function deterioration by electroretinogram (ERG) recordings in APP_{swe}/PS1ΔE9 mice. However, other laboratories failed to detect AD-associated defects. We thus sought to determine if 5.5-6-month old APP_{swe}/PS1ΔE9 mice (C57BL/6 background) presented signs of cellular degeneration and visual alterations. The expression of amyloid-derived peptides/Aβ was monitored by immunofluorescence in cryosections and by Western blotting in retinal lysates. To follow RGC survival by immunofluorescence, retinae were stained for RNA-binding protein with multiple splicing (RBPMS), osteopontin and melanopsin. Cones and rod bipolar cells were respectively labelled with peanut agglutinin (PNA), and antibodies recognizing opsins and PKCα. To study the retinal function, ERG recordings were carried out in photopic and scotopic conditions. Our results showed that amyloid/Aβ was elevated in retinal ganglion cells and in the inner nuclear layer of APP_{swe}/PS1ΔE9 compared with wild-type animals (WT). Interestingly, amyloid/Aβ immunoreactivity was especially high in OPN-positive RGCs. At this age, no amyloid plaque could be observed (n=4 mice/group). Western blot analysis revealed a higher amount of the alpha C-term fragment than that of beta C-term fragment in APP_{swe}/PS1ΔE9 samples. The number of RBPMS-labelled RGCs/section did not significantly differ between WT (278.6±7.4 RGCs, mean±SEM, n=4) and APP_{swe}/PS1ΔE9 (287.3±8.0 RGCs, n=4) mice. The number of OPN-expressing RGCs was also similar between WT (17.8±1.8, RGCs, n=4) and APP_{swe}/PS1ΔE9 (17.7±1.3 RGCs, n=4) mice. The number of M-cones was not changed in APP_{swe}/PS1ΔE9 (648.2±26.1 cells, n=4) relative to WT (614.0±17.0 cells, n=4) eyes. PNA and PKCα staining did not show obvious change in APP_{swe}/PS1ΔE9 outer retinae. The analysis of scotopic and photopic ERGs show no significant variation in a- and b-wave amplitudes in WT

(n=6) compared with APP_{swe}/PS1ΔE9 (n=5) littermates. In summary, the expression of human amyloid peptides in RGC and inner nuclear layers of APP_{swe}/PS1ΔE9 retinae do not cause cellular or functional alteration at 5.5-6 months of age. Additional experiments in older APP_{swe}/PS1ΔE9 mice will be required to determine if retinal physiology impairments arise during aging.

Disclosures: S.M. Joly: None. **S. Lamoureux:** None. **V. Pernet:** None.

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 599.08/O10

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Longitudinal cognitive testing in a mouse model of Aβ toxicity using an automated CognitionWall task

Authors: *M. LOOS[1], B. KOOPMANS[1], E. REMMELINK[1], B. R. LUBBERS[1], R. E. VAN KESTEREN[2], M. VERHAGE[2], A. B. SMIT[2];
[1]Sylics, Amsterdam, Netherlands; [2]Mol. and Cell. Neurobio., VU university, Amsterdam, Netherlands

Abstract: Alzheimer's disease (AD) is characterized by progressive neuropathological changes and decline in cognitive function. To study the consequences of Aβ oligomer-induced synaptotoxicity on cognitive function, and the efficacy of novel treatments to revert these effects, it is key to develop robust cognitive tests in mice. Here we describe the novel 1-night CognitionWall discrimination learning task, which measures cognitive function in mice in an automated home-cage. In this task, which executes without any human intervention, mice obtain their food by passing through one of three entrances in a wall placed in front of a reward dispenser. Transgenic APP/PS1 mice overproducing Aβ oligomers were significantly slower at reaching the learning criterion, not only around the age at which amyloid plaques start to be visible (26 - 30 weeks of age), but also long before plaque formation at 16 weeks of age. Moreover, the same behavioral deficit could repeatedly be observed in the same cohort tested at 17, 18 and 19 weeks of age. Several proof-of-concept interventions, including BACE1 inhibition (LY2886721), Memantine and Donepezil were used with the aim to pharmacologically validate this task. Thus, in contrast to tests such as the Morris Water Maze, which are performed once at the endpoint of an experiment (cross-sectional between-subject design), we can now longitudinally (within-subject) measure cognitive function in a single cohort of mice. Thus, the CognitionWall discrimination learning task enables longitudinal within-subject testing of AD

mouse models, it strongly reduces the number of mice required for cognitive testing in comparison to current cross-sectional studies, it is fast, and most importantly, it provides a more accurate and sensitive assessment of treatment effects in these models.

Disclosures: **M. Loos:** A. Employment/Salary (full or part-time): Sylics (Synaptologics BV). **B. Koopmans:** A. Employment/Salary (full or part-time): Sylics (Synaptologics BV). **E. Remmelink:** A. Employment/Salary (full or part-time): Sylics (Synaptologics BV). **B.R. Lubbers:** A. Employment/Salary (full or part-time): Sylics (Synaptologics BV). **R.E. van Kesteren:** None. **M. Verhage:** F. Consulting Fees (e.g., advisory boards); Sylics (Synaptologics BV). **A.B. Smit:** F. Consulting Fees (e.g., advisory boards); Sylics (Synaptologics BV).

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 599.09/O11

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Effects of extended isolation stress on amyloid beta production and cognition in 5xFAD transgenic mice

Authors: ***J. L. PETERMAN**, J. D. WHITE, M. J. EIMERBRINK, K. C. PAULHUS, M. A. THOMPSON, H. B. HAYES, G. W. BOEHM, M. J. CHUMLEY;
Texas Christian Univ., Fort Worth, TX

Abstract: The prevalence of Alzheimer’s Disease, a neurodegenerative disease characterized by the pathological hallmarks of amyloid beta (Aβ) plaques and neurofibrillary tangles, is increasing while its causes are unknown (2013 Alzheimer's Disease Facts and Figures, 2013; Hochstrasser et al., 2013). Interestingly, stress has been found to exacerbate Aβ production in transgenic mouse models of the Alzheimer’s (Devi et al., 2010; Lee et al., 2009). We hypothesized that a social stressor, isolation stress, would exacerbate amyloid beta production in 5xFAD+ transgenic mice in comparison to group-housed control animals and 5xFAD- mice. Further, it was hypothesized that isolated, 5xFAD+ animals would perform worse in contextual fear conditioning, a hippocampus-dependent memory task, than group housed, 5xFAD+ or isolated 5xFAD- animals. After extended isolation or group housing, animals underwent contextual fear conditioning, following which freezing behavior was monitored during testing 24 hours later. Twenty four hours after testing, animals were perfused and a hemisphere was collected for sectioning and staining for plaques, while the hippocampus was removed from the other hemisphere and Aβ was quantified by an Aβx-42 ELISA. The hypothesized results include significant elevations in Aβ and plaques for the isolated, 5xFAD+ animals over the group

housed, 5xFAD+ animals, both of which are significantly elevated from the isolated and group housed 5xFAD- animals. This study may help provide insight into factors that contribute to Alzheimer's, critical research when the prevalence of the disease is increasing and its causes are not yet known. Future research will explore ways to rescue these deficits.

Disclosures: J.L. Peterman: None. **J.D. White:** None. **M.J. Eimerbrink:** None. **K.C. Paulhus:** None. **M.A. Thompson:** None. **H.B. Hayes:** None. **G.W. Boehm:** None. **M.J. Chumley:** None.

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 599.10/O12

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Characterization of vascular alterations in transgenic 5xFAD mice

Authors: *E. AUER[1], K. LANEGGER[2], M. TEMMEL[1], J. NEDDENS[1], B. HUTTER-PAIER[1]; [1]Neuropharm., QPS Austria GmbH, Grambach, Austria; [2]FH Joanneum, Graz, Austria

Abstract: Accumulating evidence emphasizes the importance of neurovascular dysfunction in Alzheimer´s Disease (AD). Alterations in brain vasculature morphology and function have been reported to correlate with the progression of AD. Among these changes are microvascular atrophy, changes in blood vessel density and decreased clearance of beta-amyloid resulting in cerebral amyloid angiopathy (CAA). The astrocytic water channel aquaporin-4 has been shown to be vitally involved in the latter process.
Transgenic mouse models are frequently used to analyze the efficiency of new test compounds against AD und should thus mimic the disease as closely as possible. The present study aimed at investigating progressive vascular changes in the transgenic 5xFAD mouse model of AD by labeling 5xFAD brain tissue using antibodies directed against beta-amyloid, collagen IV as a marker for blood vessels, and aquaporin-4. The quantification of beta-amyloid over age showed increased levels in the hippocampus and cortex of transgenic 5xFAD mice compared to control littermates. This effect was first detected in 3 month old animals and became more pronounced with age. The analysis of CAA over age showed a similar progression. Furthermore, analysis of aquaporin-4 revealed that vascular-related aquaporin-4 decreased over age while parenchymal aquaporin-4 increased over age. Together, our data indicate that 5xFAD mice represent a valuable tool for studying various aspects of blood vessel dysfunction in an established AD mouse model.

Disclosures: **E. Auer:** None. **K. Lanegger:** None. **M. Temmel:** None. **J. Neddens:** None. **B. Hutter-Paier:** None.

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

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Program#/Poster#: 599.11/O13

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Deep proteome profiling of hippocampus in 5xFAD mouse model describes alterations in biological process related to Alzheimer's disease

Authors: *D. KIM[1], J. WOO[1], D. HAN[3], J. PARK[2], Y. KIM[2], I. MOOK-JUNG[1]; [2]Biomed. Engin., [1]Seoul Natl. University, Grad. Sch., Seoul City, Chongno-Gu, Korea, Republic of; [3]Biomed. Res. Inst., Seoul Natl. Univ. Hosp., Seoul, Korea, Republic of

Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disease and characterized by the deposition of extracellular amyloid plaques and intracellular neurofibrillary tangles. Although extensive research was performed for decades, the exact mechanisms and pathological causes of the disease still remain unknown. In order to perform comprehensive and integrative characterization of AD brain, assessment of global proteomic dynamics is required. We performed hippocampal proteome analysis to compare differentially expressed proteins in wild-type and 5XFAD model mice by label-free LC-MS. To reveal the relation between proteome changes and progression of amyloid plaques deposition in hippocampus, the hippocampal proteome was identified in age-dependent manner. 9,313 total proteins and 1411 differentially expressed proteins (DEP) were identified in 5 and 10-month-old wild-type and 5XFAD mice. To explore the altered biological pathway in AD mouse model, the functional and pathway-based analyses of DEP were performed by bioinformatics analysis. Using immunohistochemistry and western blot analysis, we validated novel hippocampal proteins specifically regulated in 5 and 10-month-old 5XFAD mouse model. Therefore, quantitative proteome identification of 5XFAD model mice is able to provide meaningful data to predict real biological alterations in AD and suggest novel protein biomarkers.

Disclosures: **D. Kim:** None. **J. Woo:** None. **D. Han:** None. **J. Park:** None. **Y. Kim:** None. **I. Mook-Jung:** None.

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

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Program#/Poster#: 599.12/O14

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Spatial learning and memory performance in two APP21-overexpressing transgenic rat models of Alzheimer's disease

Authors: D. KLAKOTSKAIA[1], R. RICHARDSON[1], D. BOWIE[2], S. CROSBY[2], C. JUSZCZYK[2], L. PAK[2], S. MUDD[2], C. AGCA[3], *T. SCHACHTMAN[2], Y. AGCA[3]; [1]Psychological Sci., [3]Vet. Pathology, [2]Univ. of Missouri, Columbia, MO

Abstract: Alzheimer's disease is a progressive neurodegenerative disorder that results in synaptic and neuronal loss in regions of the brain responsible for memory and cognition. To date, transgenic animal models have played a crucial role in our understanding of the underlying mechanisms of the disease. In this study, spatial memory performance in the Barnes maze was assessed in two strains of transgenic female rats that overexpress human beta amyloid precursor protein (APP21). Female rats aged 12-14 months received nine 3-min acquisition trials over the course of three days, after which 14-day retention was evaluated, and subsequently followed by three days of reversal training trials. The number of nose-poke errors made and latency to enter the target hole were recorded. There were significant group differences in performance during all three phases of Barnes maze training. It was found that both singly transgenic APP21 rats and APP21 rats with an additional presenilin 1 transgene (APP21+PS1) made significantly more errors than control Fischer rats during acquisition and reversal training. During the retention interval test, doubly transgenic APP21+PS1 rats made significantly more errors than both the control Fischer and the singly transgenic rats. These results suggest an overall spatial memory deficit in the transgenic rats and a larger spatial memory deficit in the doubly transgenic APP21+PS1 rats than the singly transgenic APP21 rats.

Disclosures: D. Klakotskaia: None. **R. Richardson:** None. **D. Bowie:** None. **S. Crosby:** None. **C. Juszczyk:** None. **L. Pak:** None. **S. Mudd:** None. **C. Agca:** None. **T. Schachtman:** None. **Y. Agca:** None.

Poster

599. Alzheimer's Disease: Models

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 599.13/O15

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Heightened emotional contagion in the mouse model of alzheimer’s disease

Authors: *J. CHOI, Y. JEONG;
KAIST, Daejeon, Korea, Republic of

Abstract: Emotional symptoms, as well as cognitive symptoms, have been found commonly in patients with Mild cognitive impairment (MCI) and Alzheimer’s disease (AD). Although cognitive deficits such as memory impairments are considered as typical symptoms of AD, emotional or neuropsychiatric symptoms are often more difficult to manage, since those symptoms can be distress and burden to their caregivers. It is well known that the patients usually showed emotional alterations, for example, depression, irritability, and agitation. In addition to those symptoms, a few researches recently suggested that they also showed high level of ‘emotional contagion’, the ability to mimic another person’s emotion unconsciously. However, the causal mechanism of the heightened emotional contagion in AD patients is still unclear.

As emotional contagion is the primitive form of cognitive empathy, it presents not only in human beings but also in rodents. Here, we carried out ‘observational fear conditioning (OFC)’, a method that can measure the level of emotional contagion in mouse model of AD to see whether they have changes in emotional contagion. Local field potential (LFP) in anterior cingulate cortex (ACC), anterior insular (AI), basolateral amygdala (BLA), and retrosplenial cortex (RSC) of the mouse brain were recorded simultaneously during the behavior and the resting state. In line with the human AD patient results, the disease model mice also showed significantly higher freezing level, which is indicating the heightened emotional contagion, compared to the littermate normal mice when they reached at the age of full symptom manifestation. Moreover, ACC in AD mice displayed significantly higher theta wave synchrony with BLA and AI, when the mice observed the suffering of others.

Thus, this study demonstrated that the heighted emotional contagion, which was reported in human AD patients, was observed in AD mouse model as well. Furthermore, the behavioral alteration had a causal relationship with neuronal synchrony within ACC, BLA and AI, which are the regions included in the salience network in the case of human brain. These results suggested a possible explanation how and why the patients with AD were easily influenced by the caregiver’s emotional state in terms of the specific brain region network connectivity.

Disclosures: J. Choi: None. **Y. Jeong:** None.

Poster

599. Alzheimer's Disease: Models

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Program#/Poster#: 599.14/O16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ARUK-IRG2014

Title: Longitudinal *In vivo* tracking of neurovascular function in a mouse model of Alzheimers disease

Authors: *J. BERWICK, P. S. SHARP, L. BOORMAN, S. HARRIS, P. HEATH, S. B. WHARTON;
Univ. Sheffield, S. Yorkshire, United Kingdom

Abstract: Neural activity is closely followed by a localised change in cerebral blood flow, a process termed neurovascular coupling. Accumulating evidence indicates that impaired neurovascular coupling may be an early pathogenic factor in Alzheimer's disease (AD), which could also serve as a perfusion-based imaging biomarker of cerebral pathology. However, there are a lack of studies examining the evolution of neurovascular dysfunction in mouse models of AD. This may be due to the challenges of controlling physiological parameters in mice and profound effects of anesthetics on vascular physiology. Our previous work has established an anesthetic regime that produces hemodynamic responses comparable to those in the awake mouse. In the present study, our aim was to adapt this protocol for use as a chronic anesthetized preparation and determine the onset and evolution of neurovascular alterations in a transgenic mouse model of AD (hAPP-J20). In addition to evoked hemodynamics, we also investigated the potential emergence and/or attenuation of low frequency hemodynamic oscillations (0.1 Hz, 'vasomotion'), which have been linked to pathophysiological conditions and could act as a disease biomarker. To visualise cerebral vasculature, mice were equipped with a cranial window and a head plate for fixation. We used optical imaging spectroscopy (OIS) to produce functional maps of changes in tissue oxygenation and cerebral blood volume in response to whisker stimulation. In addition, concurrent multi-depth electrophysiology and OIS was used to examine the relationship between neural activity and a range of hemodynamic responses. Spontaneous and evoked hemodynamics were measured every month between 3 and 12 months, which precedes the impact of vascular amyloid deposition on vessel reactivity. Our findings demonstrate the reliability of our chronic anesthetized preparation showing highly reproducible responses in the same animal over 3 months, at which time acute neural/OIS experiments were conducted. Contrary to previous reports, hAPP-J20 mice did not exhibit reductions in responses compared to controls during the first 12 months. Furthermore, vascular dynamics were also largely unchanged, however, by 9 months a larger post-stimulation undershoot emerged in

hAPP-J20 mice as a result of a rapid return to baseline. Fourier frequency analysis of spontaneous hemodynamic data, also indicated impaired 'vasomotion' in hAPP-J20 mice. Our initial findings suggest that cerebrovascular dysfunction is closely linked to vascular amyloid deposition. We will present further data exploring this possibility and include analysis of neural activity in hAPP-J20 and control mice.

Disclosures: J. Berwick: None. **P.S. Sharp:** None. **L. Boorman:** None. **S. Harris:** None. **P. Heath:** None. **S.B. Wharton:** None.

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 599.15/O17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NHMRC/ARC Dementia Research Development Fellowship APP1106751

Title: Using synthetic extracellular matrix mimics to recapitulate key pathologies of Alzheimer's disease

Authors: *A. D. MARTIN[1], L. M. ITTNER[2], Y. D. KE[2], S. CHUA[2];
[1]Chem., [2]Sch. of Med. Sci., Univ. of New South Wales, Sydney, Australia

Abstract: Two of the key neuropathologies associated with Alzheimer's disease are the formation of amyloid beta plaques and neurofibrillary tangles. Much research has been devoted to studying the formation of these key pathologies within human and animal models, including the generation of transgenic mice which overexpress the amyloid precursor protein or the tau protein. From these animal models, it has been shown that the extracellular matrix (ECM) may play a role in the fibrillogenesis of amyloid fibres, and that plaque deposition is detrimental to physiochemical properties of the ECM.
Three dimensional cell culture systems are able to offer a bridge between traditional two dimensional cell culture and animal models. Such a 3D system offers advantages from both techniques, including the high throughput associated with monolayer cell culture whilst maintaining conditions which mimic the in vivo environment found in animal models. Currently, the most common choice for creating three dimensional cell culture models is MatriGel, however it suffers from batch-to-batch variability, the presence of xenogenic components and heterogeneity. Clearly, a more reproducible, homogenous candidate is required for future 3D cell culture studies.
In this work, a series of short peptides (2-6 amino acids) bearing bulky aromatic groups at their

N-terminus have been synthesised. Upon treatment with cell culture media such as Dulbecco's Modified Eagle Medium (DMEM) or NeuroBasal, these peptides self-assemble into hydrogel networks composed of uniform, cross-linked fibres in the same manner as the extracellular matrix. Through the selection of amino acid sequence and capping group, the physical and chemical properties of these ECM mimics can be tuned, allowing the effect of parameters such as stiffness, mesh size and fibre charge on the growth of neural networks to be investigated. As such, a series of these ECM mimics have been used as three dimensional cell cultures for primary neuronal cells. The presence of the fibronectin sequence arginine-glycine-aspartic acid (-RGD) results in increased adhesion of neuronal cells to these synthetic ECM materials, with neuronal development comparable to that observed in poly-D-lysine controls. Thus it can be shown that these homogenous, reproducible hydrogel networks comprised of short peptides are able to support neuronal development. In the future, primary neuronal cells derived from transgenic mice will be cultured within these 3D systems, with the aim of concurrently recapitulating key amyloid and tau pathologies in a tuneable extracellular matrix mimic.

Disclosures: **A.D. Martin:** None. **L.M. Ittner:** None. **Y.D. Ke:** None. **S. Chua:** None.

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 599.16/O18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: GMU OSCAR Program

Title: A longitudinal investigation into the effects of Zn and Cu on spatial learning/memory and social preference in a mouse model of LOAD

Authors: *S. N. HOWELL, K. N. BOGGS, T. P. IOBST, J. M. FLINN;
Psychology, George Mason Univ., Fairfax, VA

Abstract: This study examines the effect of dietary copper (Cu) and zinc (Zn) on spatial learning/memory and social preference in a mouse model of late onset Alzheimer's disease (AD). Previous research has indicated that excess dietary Zn may cause behavioral impairments through an induced Cu deficiency. Using a mildly Cu deficient hard diet, we initially tested this theory in an early onset model of AD, which suggested instead that 1.) the effects of Cu and Zn may differ in the brain regions controlling different behaviors, and that 2.) the effect of excess Zn may not be entirely due to an induced Cu deficiency.
To examine the effects of the dietary manipulations in a late onset model, we crossed male hAPP

transgenic (Tg) mice with homozygous ApoE4 females. We are particularly interested in the E4 allele of the APOE gene, as it constitutes a high risk factor for development of AD, with some evidence showing that it may be protective early on. Six groups are being tested for spatial learning/memory (Morris water maze (MWM)) and social preference (3-chamber apparatus) at 6 and 12 months of age (wildtype (Wt) and Tg mice on (a.) lab water + Cu control diet, (b.) lab water + Cu deficient diet, or (c.) Zn-enhanced water + Cu control diet). These time points were chosen to identify longitudinal changes in behavior, allowing for identification of the potentially protective effects of the E4 allele and dietary interactions in early and late stages of AD. Open field (OF) and odor habituation/dishabituation (OHD) are being assessed as controls for the social behavior task (locomotor activity and odor sensitivity respectively).
Data at 6 months suggests early spatial learning/memory deficits but intact social preference in AD mice. AD mice showed significantly longer latencies than Wt's in the MWM ($p<.001$), but display normal preferences for social interaction in the 3-chamber test. Twelve-month data will be collected between July-November 2016 and will also be presented.

Disclosures: **S.N. Howell:** None. **K.N. Boggs:** None. **T.P. Iobst:** None. **J.M. Flinn:** None.

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 599.17/P1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR 394515

Title: Tracking of disease progression *In vivo* in a Primate Model fo Alzheimer's Disease

Authors: *R. G. WITHER[1], S. E. BOEHNKE[1], A. LABLANS[1], B. C. COE[1], J. Y. NASHED[1], D. J. COOK[1], F. G. DE FELICE[2], D. P. MUNOZ[1];
[1]Ctr. for Neurosci. Studies, Queen's Univ., Kingston, ON, Canada; [2]Inst. of Med. Biochem. Leopoldo de Meis, Rio De Janeiro, Brazil

Abstract: Alzheimer's disease (AD) is a devastating neurodegenerative disease and there is an urgent need to develop new therapeutics. Promising drugs developed in rodents have failed to work in AD patients in clinical trials. To bridge this translational gap, our laboratory has developed a non-human primate (NHP) model of AD via intracerebroventricular (icv) injection of neurotoxic amyloid beta oligomers (AβOs). This model recapitulates the molecular aspects of human AD pathology, such as tau hyperphosphorylation coupled with tangle formation, synaptic loss, and astrocytic activation (Forny-Germano et al., J.Neurosci, 2014). Here, we present results

from a pilot study tracking disease progression *in vivo* in male rhesus macaques given large icv injections of AβOs (100-400μg) every 4-6 weeks over 12-18 months. During a baseline period and across the months of injections we regularly tracked behavioural, neuroimaging and cerebrospinal fluid (CSF) and blood biomarkers. To track behavior we measured cage activity using a 3-d accelerometer activity tracker on the animal's collar and 24/7 video. To measure cognition we used a cage-side touch-screen device which implemented visuo-spatial tasks from the CANTAB AD battery (Monkey CANTAB, Lafayette). To track synaptic degradation we conducted functional magnetic resonance imaging and analyzed resting state functional connectivity. To track molecular biomarkers in the CSF we conducted lumbar punctures and used ELISA and Luminex platforms to quantify levels of Aβ1-40, Aβ1-42, total Tau, pTau and panels of cytokines. To track blood biomarkers, blood samples were analyzed regularly with glucose metabolism measured. Using the CANTAB apparatus, we observed spatial working memory deficits on the self-ordered spatial search task, and the inability to learn new tasks (delayed match to sample and paired associates learning) following AβO injections. In addition to learning and memory deficits, overall home-cage activity was diminished as assessed using 24/7 activity and video monitoring. Reductions in resting-state functional connectivity were observed among memory-related areas including the frontal cortex and hippocampus. Changes in Aβ with injections could be tracked in the CSF, along with an increase in pTau. The long-term goal is to relate these *in vivo* metrics to each animal's post-mortem brain pathology. Overall, we validate a viable platform by which to evaluate development of AD in primates, which could easily be translated to track disease in other primate models.

Disclosures: R.G. Wither: None. **S.E. Boehnke:** None. **A. Lablans:** None. **B.C. Coe:** None. **J.Y. Nashed:** None. **D.J. Cook:** None. **F.G. De Felice:** None. **D.P. Munoz:** None.

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 599.18/P2

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Generation of progranulin inducible mouse lines to determine the timeline of therapeutic intervention

Authors: *M. TOWNSEND, L. MARTENS, G. KOENIG, H. PATZKE;
FORUM Pharmaceuticals, Waltham, MA

Abstract: Frontotemporal dementia (FTD) is the second most common neurodegenerative dementing disorder and is characterized by impairments in behavior, language, and planning.

Loss of function mutations in the progranulin gene (*GRN*) are a cause of FTD. Several mouse models of progranulin (PGRN) deficiency have been generated by knocking out the *Grn* gene. Heterozygous mice, which produce approximately half the normal levels of PGRN, have been reported to exhibit a minimal phenotype at the molecular and pathological level. By comparison, knockout mice consistently show microgliosis, accumulation of lipofuscin, and altered expression of lysosomal proteins. Whether restoration of progranulin can arrest the progression of these phenotypes has not been examined.
We have generated a conditional knockin mouse model of PGRN deficiency. A stop sequence flanked by loxP sites was inserted into exon 1 of the mouse GRN genomic locus. Untreated, these mice produce no detectable PGRN in the brain. When crossed into a mouse line expressing a constitutively active CRE, progranulin levels are equal to WT. Ongoing experiments are exploring the use of stereotaxically injecting AAV-CRE to restore PGRN in discreet brain regions. Crossing the knockin mice to a CreESR1 line, should enable tamoxifen inducible excision of the stop sequence and reactivation of PGRN expression.
A second conditional knockin mouse was also developed that contains a mKate2 fluorescent tag between the signal peptide and GRNP, at the n-terminus of PGRN. The long wavelength red may prove useful in tracking the expression and distribution of mKate2-PGRN both *in vitro* and *in vivo*. Both lines are expected to be valuable tools in understanding the potential for therapeutics designed to restore PGRN to slow or reverse disease progression.

Disclosures: **M. Townsend:** A. Employment/Salary (full or part-time): FORUM Pharmaceuticals. **L. Martens:** A. Employment/Salary (full or part-time): FORUM Pharmaceuticals. **G. Koenig:** A. Employment/Salary (full or part-time): FORUM Pharmaceuticals. **H. Patzke:** A. Employment/Salary (full or part-time): FORUM Pharmaceuticals.

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 599.19/P3

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Induction of tau pathology in HEK cells recapitulates aspects of the transcriptional alternations found in human Alzheimer's disease brain

Authors: *J. N. MARCUS, K. TANIS, M. COSDEN, J. MAJERCAK, C. WINROW, J. SCHACHTER;
Merck Res. Labs, West Point, PA

Abstract: A significant hurdle in Alzheimer's disease (AD) research is the lack of translationally relevant models of disease with phenotypes similar to the pathologies found in diseased human brain. The relevance of preclinical animal models of Alzheimer's-like pathology is poorly understood and often relies on genetic mutations found in a small percentage of human AD cases. On the other hand, cellular models of AD are typically not thought to capture complex long term disease processes. We sought to determine what acute aspects of AD pathophysiology are represented in HEK293 cells overexpressing wild type or a proaggregating form of the human tau protein under control of a doxycycline inducible promoter that does not contain frontotemporal dementia mutations. In this system, tau aggregates are observed when pro-aggregating, but not wildtype, tau expression is induced. We generated genome-wide transcriptome profiles by next generation sequencing from these cells after 0, 24, or 48 hours of doxycycline induction. Significant transcriptional changes were observed in the pro-aggregating tau cell line which were distinct from those observed under conditions of overexpression of wild-type human tau. These results were compared to transcriptional modules correlating with normal aging and/or AD disease onset and progression in human brain tissue samples to determine whether any changes associated with the human disease were present in either cellular model. The presence of tau pathology in the pro-aggregating cell model recapitulated several transcriptional signatures observed in human brain tissue, whereas the wild-type tau cell line did not. These results suggest that even in the absence of the multiple cell types and chronic disease characterizing human AD, this acute cellular model of tau pathology can engage many similar cellular processes that are altered in the diseased human brain. Notably, gene expression signatures driven by changing cell type populations in AD, such as increased numbers of microglia, were not observed in the HEK model. These studies highlight the utility of transcriptional profiling in determining relevance of preclinical models and their utility for focusing on specific aspects of disease pathophysiology.

Disclosures: **J.N. Marcus:** A. Employment/Salary (full or part-time): Merck. **K. Tanis:** A. Employment/Salary (full or part-time): Merck. **M. Cosden:** A. Employment/Salary (full or part-time): Merck. **J. Majercak:** None. **C. Winrow:** A. Employment/Salary (full or part-time): Merck. **J. Schachter:** A. Employment/Salary (full or part-time): Merck.

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: SFRH/BD/88932/2012

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Portuguese North Regional Operational Program (ON.2 – O Novo Norte)

National Strategic Reference Framework (QREN)

European Regional Development Fund (FEDER)

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Title: Conditional ablation of Tau in the adult brain leads to anxious and depressive pathology

Authors: *J. M. SILVA[1,2], C. SOARES-CUNHA[2], A. RODRIGUES[2], N. SOUSA[2], I. SOTIROPOULOS[2];
[1]Life and Hlth. Sci. Res. Inst. (ICVS), Braga, Portugal; [2]Life and Hlth. Sci. Res. Inst. (ICVS), Sch. of Hlth. Sciences, Univ. of Minho and ICVS/3B's - PT Government Associate Lab., Braga/Guimarães, Portugal

Abstract: The microtubule associated protein Tau is suggested to be involved in many cellular processes such as microtubule stability, axonal transport while recent evidence suggests a role for Tau in synaptic signaling and plasticity. However, the *in vivo* significance of these Tau actions remains uncertain as conventional Tau knock-out Tau-KO) models don't exhibit the expected deficits. Overcoming the compensation mechanisms that are suggested to be involved in conventional Tau-KO models masking the real contribution of Tau protein in neuronal and brain function, we created a novel model based in a LoxP/Cre-recombinase system offering a conditional and inducible approach for studying Tau role in neuronal function and brain circuits. After an extensive characterization of the model, we demonstrate that induction of Tau deletion at 4 months old animals lead to an anxious and depressive phenotype without memory impairments. Further analysis correlates the above behavioral phenotype with morphological alterations in neurons of the prefrontal cortex (PFC) and amygdala (Amy) and electrophysioligcal coherence pointing towards an important involvement of Tau in amygdala-PFC circuitry. Moreover, current molecular analysis provides novel insights about the role of Tau in dendritic and synaptic signaling. Altogether, the loss of Tau and its function has a clear pathological impact on both neuronal structure and function in adult brain offering a new window of research that will add to our limited knowledge about the real function(s) of Tau in adult brain circuits.

Disclosures: **J.M. Silva:** None. **C. Soares-Cunha:** None. **A. Rodrigues:** None. **N. Sousa:** None. **I. Sotiropoulos:** None.

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: the Ministry of Science, ICT and Future Planning & DGIST grant 16-BD-0402

the National Research Foundation of Korean (NRF-2015M3A9E2028884)

Title: Studies of region dependent vulnerability of the olfactory system in the progress of Alzheimer's disease using tg6799 mice

Authors: *G. SON[1], S.-J. YOO[1], A. RASHEED[1], K.-A. CHANG[2], C. MOON[1];
[1]DGIST, Daegu, Korea, Republic of; [2]Gachon Univ., Incheon, Korea, Republic of

Abstract: Decreased ability of olfaction is measured prevalently in several neurodegenerative disease including Alzheimer's disease (AD). Olfactory system has a spatially conserved map depending on axon guidance when they transduce odor information. Although various attempts to define relationship which exists between olfactory circuit and AD, it has not been still fully understood how AD pathogenesis influences on abnormal behavior in olfaction. In order to provide direct correlation between olfactory dysfunction and AD, transgenic mice, Tg6799 were applied. Using immunohistochemistry, we demonstrated that loss of dopaminergic neurons in periglomerular cell by decreasing tyrosine hydroxylase and accumulation of oligomerized-amyloid beta and beta-secretases was increased in the ventral region of the olfactory bulb, as well as in the ecto-turbinate of olfactory epithelium. The immunostaining of cleaved-caspase 3 showed increase in neuronal death in the olfactory system. Next, we tried to reveal functions of the distinct odorant pathway which regions were damaged in the progress of AD. Results from real-time PCR showed decreases in expression of odorant receptors located in damaged regions. Moreover, behavioral assay demonstrated that the Tg mice lacked the response to several odorants combining with reduced receptor expression as well as behavior responses. Our results suggest that abnormal variation in the olfactory responses during AD progression may lead alternation of odor recognition pattern, as a consequence may show distinct behaviors upon odorant stimulation. Acknowledgements: This work was supported by the National Research Foundation of Korean (NRF-2015M3A9E2028884).

Disclosures: **G. Son:** None. **S. Yoo:** None. **A. Rasheed:** None. **K. Chang:** None. **C. Moon:** None.

Poster

599. Alzheimer's Disease: Models

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 599.22/P6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: University of Saskatchewan College of Medicine

Title: The locus coeruleus neurotoxin, DSP4, and/or a high sugar diet induce behavioral and biochemical alterations in wild-type mice consistent with Alzheimer's-like neurodegeneration

Authors: *L. K. BEKAR, J. PALASCHUK, P. CHOUDHARY;
Pharmacol., Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: Neurodegenerative diseases are a growing concern in our aging population. Alzheimer's disease (AD) is the sixth leading cause of death in the United States where it is estimated that one in three seniors dies with Alzheimer's or another dementia. Are modern lifestyle habits a contributing factor? Increased carbohydrate (sugar) consumption, stress and disruption of sleep patterns are quickly becoming the norm rather than the exception. Stress and high carbohydrate diets appear to be escalating diabetes and dementia cases (Alzheimer's disease is increasingly referred to as type-3 diabetes; diabetes of the brain). Interestingly, seven months on a very simple non-invasive high sucrose diet (20% sucrose in the drinking water) has been shown to induce behavioral, mitochondrial/metabolic and pathological changes consistent with AD in wild-type mice. As chronic stress and depression are associated with loss of locus coeruleus (LC) neurons and projections (source of anti-inflammatory and trophic factor control), we assessed the ability for a selective LC neurotoxin (DSP4) to accelerate and aggravate a high-sucrose mediated AD-like phenotype in wild-type mice. Male C57/Bl6 mice were divided into four groups consisting of mice that were 1) saline injected, 2) DSP4 injected, 3) given high sucrose drinking water (20%) or 4) DSP4 injected and given high sucrose drinking water. Behavioral studies at 3 months indicate that while a chronic high sucrose diet increases immobility in the forced swim test and DSP4 reduces thigmotaxis and freezing behavior in the open field test, a high sucrose diet or combined DSP4/high sucrose diet are sufficient to reduce memory in a modified Y-maze task; indicating cognitive deficits are already present 3 months after initiating treatments. The decrease in cognitive performance is consistent with an observed increase in hemibrain acetylcholinesterase activity. The process of developing a simple model in wild-type mice will highlight environmental and societal factors that need to be addressed to slow, prevent or even reverse the rising trend in national dementia patient numbers and costs.

Disclosures: L.K. Bekar: None. **J. Palaschuk:** None. **P. Choudhary:** None.

Poster

599. Alzheimer's Disease: Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Ruth K. Broad Biomedical Research Foundation (JC)

Title: Selective targeting of the corticothalamic network in an Alzheimer's disease mouse model

Authors: *R. JAGIRDAR, Y. ZHENG, J. CHIN;
Memory and Brain Res. Center, Dept. of Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Alzheimer's disease (AD) is associated with progressive memory impairment, cognitive dysfunction and a deficit in sleep maintenance. The incidence of unprovoked seizures is also higher in AD patients than in reference populations. These seemingly disparate symptoms of AD all have in common the fact that they are regulated by activity in the corticothalamic network. Transgenic mice that express human amyloid precursor protein (APP) carrying mutations linked to AD also exhibit all of these symptoms. We recently identified robust alterations in activity in the corticothalamic network in APP mice, suggesting that indeed, dysfunction in this network may be a common denominator underlying many aspects of AD pathophysiology. We found that much of the dysfunction in the corticothalamic network of APP mice appears to be downstream of a marked reduction of activity in the reticular thalamic nucleus (nRT). Such reduction was associated with impairments in sleep maintenance, deficits in hippocampal spatial memory, and seizures. We therefore hypothesized that restoration of activity in nRT might be a therapeutic strategy to improve cognition and behavior as well as reduce seizure incidence in APP mice. To do so, we are expressing Gq coupled hM3D (DREADD) receptors in nRT in both wild-type and APP mice, and then treating the mice with clozapine-n-oxide (CNO) to specifically activate DREADD-expressing nRT neurons. We demonstrate that an AAV-hM3Dq vector stereotaxically targeted to nRT reliably expresses throughout the extent of nRT. Moreover, administration of CNO to wildtype mice expressing DREADDs in nRT results in alterations in sleep maintenance, as predicted. Dose dependent responses will be evaluated in both wildtype and APP mice to study the effect on neuronal activity and sleep behavior, as well as seizures. These studies will determine whether selectively targeting the corticothalamic network might be an effective therapeutic strategy to reduce seizures and improve cognition and behavior in APP mice and possibly also in AD patients.

Disclosures: **R. Jagirdar:** None. **Y. Zheng:** None. **J. Chin:** None.

Poster

599. Alzheimer's Disease: Models

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Program#/Poster#: 599.24/P8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: GMU OSCAR program

Title: Impairments in circadian wheel running behavior and motivated behaviors in a late onset mouse model

Authors: *K. BOGGS, S. N. HOWELL, J. M. FLINN;
George Mason Univ., Fairfax, VA

Abstract: Most studies concerning mouse models of Alzheimer's disease (AD) focus on early onset AD (EOAD) due the genetic nature of the disease. These early onset cases, only account for 1-5% of total AD cases, while late onset AD (LOAD) constitutes the majority. The most well established risk factor for LOAD is the E4 allele of the apolipoprotein E gene. In an effort to model LOAD in mice, APP mutant mice (J20s) have been crossed with E4 mice to generate APP/E4 LOAD mice. The primary symptom of AD is memory loss, yet secondary symptoms are often the reason for institutionalization. Such symptoms include circadian rhythm (CR) sleep disturbances, and loss of activities of daily living (ADL). To measure ADL in APP/E4 and C57 wildtype mice, nest building and burrowing behavior were analyzed at 6 months, and will be analyzed at 12 months. These species typical behaviors in mice are likened to basic ADL in humans, which would include activities such as bathing or dressing. To assess CR sleep disturbances, wheel-running behavior was measured at 6 months and will be repeated at 12 months. For the burrowing assay, animals were individually housed 3 hours before dark onset in cages equipped with a plastic burrow filled with 250 grams of pea gravel. Burrows were weighed after 2 hours and again the following morning. At 6 months, C57 animals burrowed significantly more pea gravel overnight than APP/E4 animals ($p<.05$). For the nest-building assay, animals were individually housed for a period of 24 hours in cages lined with corncob bedding and shredded paper. At the end of the testing period, pictures of the nests were scored by blind raters. Nesting scores were lower in APP/E4 animals compared to C57 mice ($p<.05$). Taken together, APP/E4 animals performed significantly worse on both measures of ADL at 6 months of age. CR wheel running-behavior was measured using cages that track wheel rotations. Animals were individually housed in these cages for 9 days. Preliminary data suggests that APP/E4 animals have significantly altered wheel-running behavior at 6 months of age compared to controls. Measures of wheel-running behavior include: hour of onset, hour of offset, bout length, counts per bout, and bouts per day. Both 6 month and 12 month data will be presented in November.

Disclosures: K. Boggs: None. **S.N. Howell:** None. **J.M. Flinn:** None.

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 599.25/P9

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: An improved *In vitro* Blood Brain Barrier model including primary neurons for Alzheimer Disease high content screening

Authors: D. BUTTIGIEG[1], *B. B. DOROTHEE[1], Y. MOLINO[2], E. GRAS-LAVIGNE[1], H. HENRIQUES[1], F. JABES[2], M. KHRESTCHATISKY[3], R. STEINSCHNEIDER[1];
[1]Neuron Experts, Marseille, France; [2]Vect-Horus, Marseille, France; [3]NICN-UMR7259, AMU CNRS, Marseille, France

Abstract: Alterations of the Blood-Brain Barrier (BBB) are likely involved in several neurodegenerative diseases. The BBB is a dynamic interface that doesn't only react to vascular signals but also produces prostaglandins, nitric oxide, and cytokines that affect Central Nervous System (CNS) function through its detoxification and anti-inflammatory capacities. Conversely, cerebral capillaries are innervated by different noradrenergic, serotonergic, cholinergic or GABA-ergic neurons that regulate important aspects of BBB function and induce the expression of BBB-related proteins. Several studies argue that dysfunction of BBB precipitates Alzheimer Disease (AD). Faulty BBB clearance of A-beta (Aβ) may promote its accumulation in the brain. Additional features of AD such as angiogenesis and arterial dysfunction may also initiate neurovascular uncoupling leading to Aβ accumulation and neurovascular inflammation resulting in synaptic and neuronal dysfunction. Barrier alteration in neurodegenerative diseases also has significant implications for drug therapy. Numerous drugs weren't tested because preliminary studies suggest they don't cross the BBB despite the fact that they could benefit from increased brain delivery across a compromised BBB. The study of the dynamic nature of BBB, its dysfunction and the potential involvement of the peripheral compartment in neurodegenerative processes may help to better understand the pathophysiology of CNS disease. We developed an improved *in vitro* rat syngeneic BBB model that includes primary cortical neurons. First, we established in 12 well plates the best conditions of culture between astrocytes, cortical neurons and microvascular endothelial cell monolayers. We characterized each cell partners by phenotypical features and specific immune-labelling (Claudin-5, ZO-1, MAP2, GFAP…) before investigating integrity of the monolayers by quantification of endothelial permeability coefficient (Pe) for lucifer yellow (LY). Then we miniaturized the model in 96 well plates for high content screening and applied oxidative stress damage (glutamate injury) and Aβ intoxication, two processes involved in AD. We showed the responsiveness of our cell system and the implication of each cellular counterpart in the neurodegenerative events. Finally, we tested some reference compounds and demonstrated the robustness of this neuro-glio-vascular model. This new

relevant BBB cell model based on co-culture of three types of primo-cultures will allow a more predictive evaluation of therapeutic compounds.

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Poster

599. Alzheimer's Disease: Models

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Program#/Poster#: 599.26/P10

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Next-generation neurological disease models - Directed differentiation of iPSCs to functionally model Alzheimer's and Parkinson's diseases

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Abstract: Alzheimer's and Parkinson's diseases are incurable and debilitating neurodegenerative conditions with strong links to age. Today Alzheimer's disease alone accounts for 60-70% of dementia cases and Parkinson's disease is expected to affect ~2% of individuals over 65 years. The human and economic impact of these conditions is expected to increase significantly with the increasingly aging global populations unless new therapeutic strategies can be developed. Currently, there are a lack of human cell-based models available in which to carry out such studies and further investigation is needed in order to elucidate disease mechanisms and determine the efficacy of novel drug compounds. iPSC-derived neural stem cells (NSCs) offer a virtually unlimited source of physiologically relevant isogenic lines for use in both disease modelling and drug discovery.Combining the powerful tools of iPSC genome editing using CRISPR-Cas9 and directed differentiation, we have generated patient relevant NSC disease models carrying Alzheimer's disease-associated microtubule-associated protein TAU (MAPT) mutations, R406W, P301L and V337M and Parkinson's disease-associated leucine-rich repeat kinase 2 (LRRK2) mutation, G2019S in both a heterozygous and homozygous manner. These clinically identified missense mutations in MAPT are thought to reduce the ability of TAU to promote microtubule assembly and may contribute to neuronal death in Alzheimer's disease. LRRK2 is thought to contribute to Parkinson's disease via pathological mechanisms involving TAU, oxidative stress, α-synuclein, and mitochondrial-synaptic-dysfunction. Genome-edited

iPSC lines were genotyped, karyotyped and subsequently differentiated using fully-defined, xeno-free neural induction conditions (Shi et al., 2012). Once the cells formed polarized neural tube-like rosette structures in monolayer culture, immunocytochemistry confirmed the expression of typical cerebral cortical NSC markers namely, PAX6 and FOXG1. Electrical activity was confirmed used multi-electrode array (MEA).These genetically defined, functionally validated human iPSC-derived NSCs provide a renewable resource of disease- and biologically-relevant cells. These cells, carrying relevant mutations, offer a stable platform on which novel therapeutic agents can be screened and validated. Furthermore, these cells enable a direct comparison of the variant effect on cellular phenotype between isogenic lines cells and may therefore provide further insight into the pathology of these diseases.

Disclosures: **A. Armesilla-Diaz:** A. Employment/Salary (full or part-time): Horizon Discovery. **R. Sharma:** A. Employment/Salary (full or part-time): Axol Bioscience. **R. Santos:** A. Employment/Salary (full or part-time): Horizon Discovery. **B. Newman:** A. Employment/Salary (full or part-time): Axol Bioscience. **K.M. Gamber:** A. Employment/Salary (full or part-time): Horizon Discovery. **G. Gibbons:** A. Employment/Salary (full or part-time): Axol Bioscience. **C.L. Schofield:** A. Employment/Salary (full or part-time): Horizon Discovery. **N. Carpenter:** A. Employment/Salary (full or part-time): Axol Bioscience. **C.E. Lowe:** A. Employment/Salary (full or part-time): Horizon Discovery. **Y. Shi:** A. Employment/Salary (full or part-time): Axol Bioscience.

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 599.27/P11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ARDRAF 12-6 to AE

UVA Harrison Award to SC

Title: Ultrastructural neuropathology at pre-plaque ages in triple transgenic mouse model of Alzheimer’s disease

Authors: S. CHAWLA, L. HANLEY, E. E. MAHER, *A. ERISIR;
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Abstract: Amyloid plaques, the major anatomical diagnostic criteria for Alzheimer’s Disease (AD), manifest decades before clinically diagnosed dementia, which correlates more directly

with neurofibrillary tangles and neuron degeneration. A similar symptom mismatch is observed in transgenic mouse models of the disease: behavioral deficits become evident months before any extracellular amyloid accumulation or cell death occurs. In contrast, myelin deficits are the earliest neuropathological findings detected both in human familial AD patients and in transgenic mouse models. In the triple transgenic mouse model (3xTg), behavioral defects, white matter disruptions, and changes in myelin marker expression are all evident as early as 3–4 months of age. We used electron microscopy to document early neuropathological alterations in 3xTg mice at postnatal months (PM) 3, 5, 8, and 12. While non-toxic A11 fragments were detected intracellularly in hippocampal neurons of young animals, the organelle and fibril morphology in labeled neurons were not notably altered. Similarly, extracellular $A\beta_{42}$ labeling, nor plaques, were not robust before PM12. Early signs of neuronal aging are evident at PM8, in form of vacuoles, lipofuscin accumulations and tight fibril bundles in karyoplasm, protoplasm and dendrites. While synapse density was minimally altered in the hippocampus, small dendrites and terminals in the basal forebrain were notably damaged by PM5, despite unaltered cell bodies and large dendrites. The earliest neuropathological manifestations were found in myelinated axon tracts. Several abnormal myelin structural formations were encountered, including vacuolization and uncompacting of myelin stacks, ruffling of oligodendrocyte membranes, herniation, double myelin, extensive myelin outfolding and myelin bulbs. While pathological morphologies associated with oligodenrocytes progressed in severity and frequency by age, most axons surrounded by altered myelin formations, including myelin bulbs, displayed relatively intact axon morphology, indicating that oligodendrocyte pathology preceded axonal degeneration. Subtle myelin outfolding encountered at PM3 progressed to extensive hypermyelination of non-axonal neuropil and myelin bulbs, leading degeneration of sequestered neuronal elements. Our results highlight the importance of alterations in oligodendrocyte function as the earliest neuropathology in the 3xTg mouse model, indicating that oligodendrocyte pathology may be responsible for deficits in neuronal function and behavior that occur in transgenic animals prior to amyloid and tau-associated pathologies.

Disclosures: **S. Chawla:** None. **L. Hanley:** None. **E.E. Maher:** None. **A. Erisir:** None.

Poster

599. Alzheimer's Disease: Models

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Program#/Poster#: 599.28/P12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: (CONACYT Grant No: 219703)

Title: Increase in beta-sheet secondary structures of peptides associated to amyloid beta 1-42 expression in hippocampal rats exposed to ozone

Authors: *S. L. RIVAS-ARANCIBIA[1], E. RODRIGUEZ-MARTÌNEZ[2], U. LÓPEZ-GONZÁLEZ[3], I. BADILLO-RAMÍREZ[4], J. M. SANIGER[5];
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Abstract: The aim of this work was to study the effect of oxidative stress on the changes of the secondary structure (alpha-helix or beta-sheet) of peptides. These changes could be associated to amyloid beta 1-42 peptide (AB 1-42) expression in dentate gyrus of rats exposed to low ozone doses, during different periods of time. Thirty-six animals were divided in 6 groups (n=6) exposed to ozone-free air stream (control group) and to ozone (0.25 ppm) during 7, 15, 30, 60, and 90 days respectively. After the rats were deeply anesthetized and processed by the following technics: Raman spectroscopy and immunohistochemistry against AB 1-42. Raman spectroscopy was performed on the hippocampal dentate gyrus of one rat per group and 20 single spectra per group were acquired. Results of the deconvolution of Amide I in the Raman spectra showed that exposure to ozone decreased the percentage of alpha helix secondary structure of peptides as it follows: control group (60%), 7 days (47%), 15 days (46%), 30 days (41%), 60 days (22%) and 90 days (20%). On the other hand the percentage of beta folded sheets increased compared with the control groups as it follows: control group (0%), 15 days (16%) 30 days (23%), 60 days (52%) and 90 days (48%). Immunohistochemistry results showed an increase of intracellular immunoreactivity to amyloid beta 1-42 at 60 and 90 days of exposure to ozone compared with the control group. In conclusion, we found that oxidative stress caused by low doses of ozone induced an increase of beta-sheet secondary structure of peptides, which is associated with an increase in intraneuronal formation and deposition of beta-amyloid 1-42 in hippocampal dentate gyrus of rats exposed to this gas. This could be similar to what happens in patients with Alzheimer's Disease. This work was supported by the CONACYT [Grant N^{o}: 219703 to S.R.-A.].

Disclosures: S.L. Rivas-Arancibia: None. **E. Rodriguez-Martìnez:** None. **U. López-González:** None. **I. Badillo-Ramírez:** None. **J.M. Saniger:** None.

Poster

599. Alzheimer's Disease: Models

Location: Halls B-H

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Program#/Poster#: 599.29/Q1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: KAKENHI 22221004

KAKENHI 15K15085

Title: Comparative gene expression profiling of triple-transgenic 3xTg-AD and APP-knock-in model mice of Alzheimer's disease

Authors: *E. CASTILLO[1], J. LEON[1], N. ABOLHASSANI[1], G. MAZZEI[1], K. SAKUMI[1], T. SAITO[2], T. SAIDO[2], Y. NAKABEPPU[1];
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Abstract: Alzheimer's disease (AD) is the major type of senile dementia, but there is no successful treatment until now due to its complexity. To better understand the molecular mechanisms underlying AD pathology, several mouse models have been developed to mimic the main neuropathological hallmarks. In the present study, we performed a comparative gene expression profiling of two different AD model mice: the classical triple-transgenic model mice 3xTg-AD which carry two mutated human transgenes, *APP* (Swedish KM670/671NL) and *MAPT* (P301L) driven by exogenous *Thy*1.2 promoter with a knock-in mutation of *Psen1* (M146V) that promotes formation of plaques and tangles; and the recently established AD model mice APP NL-G-F with three pathogenic *App*-knock-in mutations (Swedish KM670/671NL, Arctic E693G and Iberian I716F) that promote aggressive amyloidosis under the control of endogenous *App* promoter. We prepared total RNA from cortex of 12-month-old male mice of each AD model and control, obtained their gene expression profiles using microarray technology and performed a comparative analysis. In both AD models, we observed no significant reduction in expression levels of neuronal markers, but genes involved in neuroprotection (*Fos, Nr4a1, Nr4a2, Nr4a3, Egr2*) were commonly downregulated. Among 306 significantly altered genes in the 3xTg-AD cortex (fold change < -1.2 or > 1.2, ANOVA $p < 0.05$), we found genes related to neurological diseases (21), metabolic disorders (21) and immune response (17). In contrast, among 257 altered genes in the APP NL-G-F cortex, the most significantly upregulated genes encode proteins involved in the inflammatory (18) and immune (18) responses (top 5: *Cst7, Clec7a, Ccl3l3, Lilrb4*), accompanied by a significant upregulation of astrocyte markers (*Aqp4*, *Gfap*, *S100b*) and genes encoding complement components (*C1qa, C1qb, C1qc, Tyrobp*), as well as alterations in genes related to neurological disease (18) and lipid metabolism (13). The results

observed in APP NL-G-F mice suggest that amyloidosis induced by endogenous expression of pathogenic APP protein may cause strong inflammatory/immune responses that would play important roles in AD pathogenesis. Recent reports highlight the contribution of astrocytic activation to neuroinflamation in AD, however, the mechanism involved is still unclear. To delineate the astrocytic activation by endogenous β-amyloid, we are now examining the gene expression profiles, biochemical and physiological properties of cultured astrocytes isolated from adult APP NL-G-F.

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Poster

599. Alzheimer's Disease: Models

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Program#/Poster#: 599.30/Q2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 6930217

6930217

Title: Morphological characterization of glia reveals spatiotemporal changes in Alzheimer's disease pathology

Authors: *F. ABDURROB, R. CANTER, K. CHUNG, L.-H. TSAI;
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Abstract: Glia play a crucial role in maintaining homeostasis in the brain and supporting the functioning of neurons. A complex network of inflammatory and neurodegenerative responses, coupled with amyloid beta deposits, tau tangles, and oxidative stress characterize Alzheimer’s disease. Activation and proliferation of microglia and astrocytes are important in the inflammatory response, which ultimately affects disease progression. Consequently, the synergistic changes that occur during gliosis have become an intense topic of investigation, but remain poorly defined. This study utilizes 5xFAD mice, as well as healthy age-matched control animals, to spatiotemporally characterize glial cells in the brain as aging occurs. Cutting-edge CLARITY and SWITCH labeling techniques are used to visualize the cellular landscape of the diseased brain. Morphological and proliferative changes in microglia and astrocytes are observed across brain regions as Alzheimer’s disease progresses and amyloid plaque load increases. The

interaction of these immune cells with the vasculature is also examined to explore potential impacts on amyloid clearance. Ultimately, a clearer picture of the glial changes during disease progression will allow for a better understanding of the dynamic pathophysiology that underpins neurodegeneration. Funding Sources: NIH (cost object 6930217) and the Glenn Foundation.

Disclosures: **F. Abdurrob:** None. **R. Canter:** None. **K. Chung:** None. **L. Tsai:** None.

Poster

600. Alzheimer's Disease: In Vitro Therapeutics

Location: Halls B-H

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Program#/Poster#: 600.01/Q3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Yonsei University Future-leading Research Initiative (Yonsei Challenge) of 2015 (2015-22-0137)

Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2015R1C1A1A02036851)

Title: Non-invasive intracranial mesenchymal stem cell transplantation with low intensity focused ultrasound: preliminary results of energy dependent transplantation efficiency

Authors: *J. LEE[1,2], J. SHIN[1,2], C. KONG[2], B.-W. SONG[3,4], B.-S. KIM[3,4], J. CHANG[1,2], W. CHANG[2];
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Abstract: Introductions
Stem cell therapy for neurodegenerative diseases has been reported with promising results through neuronal differentiation or control of microenvironment. However, the surgical transplant methods such as parenchymal or intravenous injection has limitations of secondary injury, infection and low survival rate of stem cell in the target site. Therefore, this study investigates a possibility of focused ultrasound (FUS) for targeted non-invasive stem cell transplantation into the brain.
Materials and Methods
In this study, Male Sparague-Dawley rats (300-350g) and bone marrow-derived mesenchymal

stem cell (BM-MSC; 5p) were used. Experimental groups consist of low intensity FUS+MSC, MSC-only and FUS+dye (Evans blue or Light green). All rats were anesthetized with ketamine cocktail, and FUS was applied with parameters of 1Mpa, 300s (AP+0.7, ML+2, DV-5.5). Three hours after sonication, BM-MSC was injected into the tail vein.

Results

First, the position of BBB opening was confirmed by the FUS+dye group. Comparing FUS+MSC and MSC-only group, it was confirmed that FUS increase BM-MSC homing to the sonicated brain tissue. Also, we found that location stained with Evans blue, a dye with large molecular weight, had a higher efficacy of stem cell homing compared to the location stained with Light green, a dye with smaller molecular weight. On the other hand, BM-MSC was not shown in group of MSC-only.

Conclusions

As a result, we confirmed MSC can be delivered into the brain by FUS. And the efficiency of stem cell delivery may be positively correlated to the ultrasonic energy. This study can contribute to determination of optimal energy for repetitive FUS to cell transplantation without tissue damage in neurodegenerative diseases. Further study about function of stem cell transplanted in brain and mechanisms of stem cell homing by low intensity FUS is needed.

Acknowledgement

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Poster

600. Alzheimer's Disease: In Vitro Therapeutics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 600.02/Q4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MRC

Title: A novel $GABA_A$ alpha5 subunit inverse agonist as a potential memory enhancer

Authors: *A. B. ALI, A. KHAN, A. MONACO, M. KUTA, M. NICHOLSON, S. HAIDER, J. JOVANOVIC, S. HILTON;
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Abstract: Short-term memory deficits are the first symptom of the late-onset Alzheimer's disease. This progressive disease constitutes one of the most significant health problems confronting mankind in societies with an aging population. Current medication for neurodegenerative disorders has only modest therapeutic efficacy. To tackle these problems with committed focus, we developed a novel molecule with enhanced bio-availability and target specificity with the potential to restore/halt memory deficits.
Using medicinal chemistry and computational modelling combined with site-directed mutagenesis we have developed hybrids of 6,6-dimethyl-3-(2-hydroxyethyl)thio-1-(thiazol-2-yl)-6,7-dihydro-2-benzothiophen-4(5H)-one, that demonstrated selective binding and high inverse agonism for the alpha5 $GABA_A$ receptor subtype. We have designed analogues of this compound with an array of biological activity ranging from inactive controls to highly potent derivatives. Whole-cell electrophysiological recordings performed provide evidence to suggest that one of the hybrids developed, a5AM21 shows better potency compared to other published analogues of this compound at specific interneurons that mediate inhibition via postsynaptic alpha 5 containing $GABA_A$ receptors. Behavioural studies performed using healthy rodents suggest that a5AM21 improved memory recall in vivo. Furthermore, this observation was consistent with in vitro electrophysiology experiments that demonstrated an enhanced hippocampal long-term plasticity at unitary synaptic recordings performed between specific dendrite-targeting interneurons.
In summary, using a multi-disciplinary approach we have developed a selective and potent potential cognitive-enhancer, a5AM21. This compound showed properties of selective inverse agonist for the alpha5 $GABA_A$ receptor subtype, which is important for inhibitory neurotransmission and memory formation.

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Poster

600. Alzheimer's Disease: In Vitro Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG043415

NIH Grant NS049442

Title: *In vivo* function of a novel and highly selective 5HT2b receptor antagonist devoid of agonist activity

Authors: *O. ARANCIO[1], J. P. SCHAVOCKY[3], S. M. ROY[3], L. CHICO[3], A. STANISZEWSKI[2], D. M. WATTERSON[3];
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Abstract: G protein-coupled receptors (GPCRs) are a well-established therapeutic target class that continues to be an area of commercial drug development and active basic science research in neurosciences. A major challenge has been the need for more selective antagonists devoid of agonist activity for a given GPCR. The goal is to avoid adverse clinical events while providing a more rational basis to therapeutic intervention. The challenge has been especially true for the 5HT2b receptor with mixed agonist/antagonist drugs being withdrawn from the market due to adverse events yet mixed GPCR antagonists low in agonist function having attractive pharmacological actions.
We report here the discovery of a novel, highly selective 5HT2b receptor antagonist, MW01-8-071HAB (MW071) that is devoid of agonist activity and rescues associative memory defect in an Alzheimer's disease (AD) relevant animal model. MW071 was synthesized as part of the experimental validation of an expert systems approach to risk reduction in drug metabolism focused on CYP2D6 substrate status, a significant contributor to individual variance in drug efficacy or toxicity (Chico et al., Drug Metab Dispos. 37:2204-2211, 2009). Remarkably, a single atom change resulted in a CYP2D6 substrate to non-substrate status. However, a recurrent theme in the discovery and development of new GPCR drug candidates is the finding that small changes in a GPCR ligand can result in significant changes in receptor modulation and in vivo function. Therefore, we took MW071 through large-scale functional screens for GPCR antagonist and agonist activities, which revealed that MW071 is a selective 5HT2b receptor antagonist that lacks detectable 5HT2b receptor agonist activity. We also subjected MW071 to large-scale hierarchical kinase inhibitor screens for potential non-GPCR ligand activity that could potentially influence GPCR mediated cellular function. Taken in its entirety, the data are consistent with MW071 being a new highly selective 5HT2b receptor antagonist that lacks both CYP2D6 substrate and 5HT2b receptor agonist liabilities capable of providing new insights into modulation of AD related cognitive dysfunction. Overall, the results add to the growing database of novel GPCR receptor modulators with new in vivo functions through the use of dual agonist/antagonist functional screens as a primary screen in drug discovery.

Disclosures: O. Arancio: None. **J.P. Schavocky:** None. **S.M. Roy:** None. **L. Chico:** None. **A. Staniszewski:** None. **D.M. Watterson:** None.

Poster

600. Alzheimer's Disease: In Vitro Therapeutics

Location: Halls B-H

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Program#/Poster#: 600.04/Q6

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Using high throughput screening method to identify new drugs for Alzheimer’s disease

Authors: *B. ZHU, K. HERRUP;
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Abstract: Aging is the major and primary risk factor of Alzheimer’s disease (AD). In the aged brain, oxidative stress, DNA damage, chronic inflammation and other stressors increase, making neurons more susceptible to abnormal cell cycle re-entry, and further leading to neuronal loss. Thus, while each of these stressors could be studied individually, blunting the effects of aging may be the most economical strategy to fight Alzheimer’s disease. To screen for drugs that have anti-aging effects, we turned to the deep ocean, which has high bacterial biodiversity and an even broader spectrum of natural bioactive compounds. We report here our successful first use of this rich source of potential drugs. To create an *in vitro* model of aging, we use mouse primary cortical neuronal cultures on both day *in vitro* (DIV) 14 and DIV 21, which we consider to mimic mature and old states respectively. The level of the G1 phase cell cycle marker, cyclin D1, increased significantly from DIV 14 to DIV 21, indicating that with age primary neurons undergo cell cycle stress. We found further that fibrillarized amyloid-beta ($A\beta_{1\text{-}42}$), increased the cyclin D1 level at both time points, suggesting that Aβ exacerbates the cell cycle stress and does so at all ages. We used fibrillar Aβ as the stimulant and cell cycle markers as outcome measures to determine whether any marine bacterial extract might prove neuroprotective. For comparison we also used previously characterized compounds from single bacterial species as well as other marine sources such as fish oil and PCSO-524®, an extract of mussel. We used in cell western methods to measure the total level of cell cycle, synaptic density, senescence marker and other response proteins to evaluate the response to fibrillar Aβ. To make the anti-aging screening assay more efficiently, we applied the screening in a 96-well format. Using this method, we discovered significant neuroprotective activity in several of the 280 compounds we have screened. As reported previously, fish oil and PCSO-524® both significantly lowered the levels of cyclin D1 and other stress-related proteins. We will also report the results of chronic incubation of these agents. Our hypothesis is that any agent that lowers stress markers compared to control has potential to blunt the effects of aging in our system. We believe that both the novel source of natural products and the effective and efficient new screening method will be of significant value in the search for new compounds to fight aging and Alzheimer's Disease.

Disclosures: B. Zhu: None. **K. Herrup:** None.

Poster

600. Alzheimer's Disease: In Vitro Therapeutics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 600.05/Q7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NRF-2013R1A2A2A01067761

Title: MSCs inhibit transmission of amyloid beta through lipid raft-mediated endocytosis.

Authors: *Y. JUNG[1,2], S. OH[1,2], P. LEE[1,2,3];
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Abstract: A hallmark pathological feature of Alzheimer's disease (AD) is the accumulation of extracellular plaques composed of the amyloid-beta (Aβ) peptide. Aβ is propagated by cell-to-cell transmission and they affect the onset and progression of AD. To block Aβ transfer is important therapeutic strategies against AD. Using Aβ enriched models, we found that mesenchymal stem cells (MSCs) contribute to the reduction of Aβ endocytosis. And we found that MSCs inhibit Aβ transmission by blocking the lipid raft-mediated endocytosis of extracellular Aβ via modulation of the interaction with lipid raft associated proteins, which led to a prosurvival effect on neurons with functional improvement of synaptic damage and cognitive decline in Aβ-enriched models. The present data indicated that MSCs exert neuroprotective properties through inhibition of extracellular Aβ transmission, suggesting that the property of MSCs may act as a disease-modifying therapy in AD. Acknowledgments : This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Science, ICT and Future Planning (NRF-2013R1A2A2A01067761), and the Brain Korea 21 PLUS Project for Medical Science, Yonsei University.

Disclosures: **Y. Jung:** None. **S. Oh:** None. **P. Lee:** None.

Poster

600. Alzheimer's Disease: In Vitro Therapeutics

Location: Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The Shoemaker Award for Neurodegenerative Research

NIH Grant 2R01 NS034239

NIH Grant 1P01 DA028555

Title: The mixed-lineage kinase 3 inhibitor URMC-099 facilitates microglial amyloid-β phagolysosomal degradation

Authors: *T. KIYOTA[1], W. DONG[1,3], C. M. EMBURY[1], Y. LU[1], W. M. WHITMIRE[1], B. DYAVARSHETTY[1], H. A. GELBARD[4], H. E. GENDELMAN[1,2];
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Abstract: Amyloid-β (Aβ)-stimulated microglial inflammatory responses directly affect Alzheimer's disease (AD) progression. Such responses, in a measure, are orchestrated through mitogen-activated protein kinase (MAPK) signaling pathways. The mixed-lineage kinases (MLKs) are known to regulate MAPK pathways including c-Jun amino-terminal kinase (JNK) and p38 MAPK. However, whether MLK-MAPK participates in Aβ-mediated neuroinflammation is poorly understood. To this end, we used URMC-099, a brain penetrant small-molecule MLK type 3 (MLK3) inhibitor, to study its effect on Aβ-mediated microglial inflammatory responses. Murine microglia were stimulated with Aβ1-42 (Aβ42), and the phosphorylation of p38 (p-p38) and JNK (p-JNK) and the expression of pro-inflammatory cytokines interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α were investigated. While Aβ42 stimulation enhanced p38, JNK phosphorylation and the expression of IL-1β, IL-6 and TNF-α, URMC-099 treatment reduced their phosphorylation and expression levels. URMC-099 induced gene expression of anti-inflammatory cytokines *IL-4* and *IL-13*. Furthermore, URMC-099 modulated scavenger receptor expression and facilitated Aβ uptake and degradation through endolysosomal Aβ trafficking. These data suggest a new immunomodulatory role for URMC-099 modulating microglial activation through MLK-MAPK with concomitant M2 microglial polarization. Thus, the MLK3 inhibitor may be considered as a novel disease-modifying AD therapy.

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Poster

600. Alzheimer's Disease: In Vitro Therapeutics

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 600.07/Q9

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Engineering novel General Amyloid Interaction Motif (GAIM)-immunoglobulin fusions for targeting misfolded protein aggregates in neurodegenerative diseases.

Authors: *M. PROSCHITSKY, E. ASP, C. CHUNG, J. LEVENSON, H. TSUBERY, M. LULU, S. GILEAD, M. GARTNER, S. SCHROETER, J. WRIGHT, R. FISHER, R. KRISHNAN;
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Abstract: We have previously identified a General Amyloid Interaction Motif (GAIM) that binds and remodels a variety of fibrillar aggregates in a conformation-dependent manner (Krishnan et.al, 2014). This unique protein scaffold was derived from the tip protein g3p of the filamentous bacteriophage M13. To make an acceptable drug candidate, we genetically fused GAIM with a human immunoglobulin (hIgG1Fc). The fusion protein, NPT088, displays bivalent GAIM from human Fc, and when injected intraperitoneally, significantly reduces Aβ plaques in the brains of Tg2576 mice, hyper phosphorylated and fibrillary tau in rTg4510 mice and ameliorates cognitive deficits associated with both the animal models (Levenson et.al, under review). To understand the molecular mechanism facilitating aggregate binding and clearance, we performed extensive mutagenesis of GAIM. The binding properties of GAIM-variants to different amyloid fibers were studied using SPR, ELISA and fiber remodeling assays. Specific binding and remodeling are strongly influenced by GAIM valence, appropriate exposure of non-polar and aromatic residues in the binding sites, and the solvated stability of the molecule as a whole. We used these data and molecular modeling tools to engineer new GAIM-fusions. Here we present data on one such fusions, NPT289, that binds multiple amyloid fibers with 5-100 fold greater potency than NPT088, remodels Aβ aggregates, and blocks cell-to-cell transmission of α-synuclein and tau aggregates in primary neuronal cells more efficiently than NPT088. Characterization of NPT289 and other GAIM variants has begun to elucidate the complex

structure activity relationship of GAIM and as a result, has created a platform for discovery of new drug candidates for protein misfolding diseases.

Disclosures: **M. Proschitsky:** A. Employment/Salary (full or part-time): Neurophage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurophage Pharmaceuticals. **E. Asp:** A. Employment/Salary (full or part-time): Neurophage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurophage Pharmaceuticals. **C. Chung:** A. Employment/Salary (full or part-time): Neurophage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurophage Pharmaceuticals. **J. Levenson:** A. Employment/Salary (full or part-time): Neurophage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurophage Pharmaceuticals. **H. Tsubery:** A. Employment/Salary (full or part-time): Neurophage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurophage Pharmaceuticals. **M. Lulu:** A. Employment/Salary (full or part-time): Neurophage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurophage Pharmaceuticals. **S. Gilead:** A. Employment/Salary (full or part-time): Neurophage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurophage Pharmaceuticals. **M. Gartner:** A. Employment/Salary (full or part-time): Neurophage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurophage Pharmaceuticals. **S. Schroeter:** A. Employment/Salary (full or part-time): Neurophage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurophage Pharmaceuticals. **J. Wright:** A. Employment/Salary (full or part-time): Neurophage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurophage Pharmaceuticals. **R. Fisher:** A. Employment/Salary (full or part-time): Neurophage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurophage Pharmaceuticals. **R. Krishnan:** A. Employment/Salary (full or part-time): Neurophage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurophage Pharmaceuticals.

Poster

600. Alzheimer's Disease: In Vitro Therapeutics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 600.08/Q10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: VA Grant NURD-016-13F

Title: Lithium treatment suppresses IP_3-gated calcium signaling and synaptic plasticity in the hippocampus of 3xTg-AD mice

Authors: *S. S. SHIM[1], N. KAPECKI[2], C. BRIGGS[3], G. E. STUTZMANN[3];
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Abstract: Although lines of evidence suggest that lithium, a CNS-acting element using for mood stabilization, may be beneficial for treating Alzheimer's disease (AD), the mechanisms underlying its therapeutic potential are not well understood. To examine the effects of lithium on AD-related pathophysiology (intracellular Ca^{2+} responses, neurophysiology and synaptic plasticity), whole-cell patch clamp recording, UV photolysis of caged-IP_3, and 2-photon Ca^{2+} imaging were conducted in CA1 hippocampal pyramidal neurons from control (NonTg) and 3xTg-AD mouse models (~3 months of age). In control mice, IP_3-evoked Ca^{2+} responses were elevated in 3xTg-AD neurons (100 ± 18% over baseline, n=18) compared to NonTg neurons (68 ± 16% over baseline, n=16). Feeding with a lithium chow diet (0.2%, Harlan Lab.) for one month had no effect on NonTg IP_3-Ca^{2+} responses (68 ± 24% over baseline, n=14) but significantly reduced the IP3-Ca^{2+} response in 3xTg neurons by almost 50% (n=15, $p < 0.05$). Despite VGCC responses not being elevated in 3xTg-AD neurons, lithium treatment also suppressed action potential-evoked Ca^{2+} responses, with no effect on NonTg VGCC responses. We also measured synaptic transmission and plasticity at the CA3-CA1 Schaffer collateral synapse using field potential recordings in hippocampal slices from the same population of 3xTg-AD and NonTg mice. Lithium treatment had no significant effects on long-term potentiation (LTP) in either mouse strain, nor did it affect passive membrane properties such as resting membrane potential or input resistance. However, post-tetanic potentiation (PTP), a form of short-term plasticity reliant on calcium signaling, was significantly increased in lithium-treated NonTg mice by almost 70% ($p<0.05$); notably, lithium had no effect on PTP in 3xTg-AD mice indicating an alternative calcium signaling mechanism is recruited in the AD model. Thus, lithium treatment suppresses activity-dependent Ca^{2+} signaling and fails to potentiate PTP in 3xTg-AD mice, which may reflect its diverse actions on IP_3 receptor stabilization, kinase activity, and Na^+/Ca^{2+} exchanger properties. Based on these early findings, the extent of neuroprotection offered by lithium in AD models warrants further study.

Disclosures: **S.S. Shim:** None. **N. Kapecki:** None. **C. Briggs:** None. **G.E. Stutzmann:** None.

Poster

600. Alzheimer's Disease: In Vitro Therapeutics

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Program#/Poster#: 600.09/Q11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NRF-2011-00503

NRF-2012R1A5A2A28671860

Title: Neuroprotective effects of polydatin on amyloid beta$_{25-35}$-induced neuronal and cognitive dysfunction through inhibition of apoptosis signaling pathway

Authors: ***Y.-H. KO**[1], S.-Y. LEE[2], C.-G. JANG[2];
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Abstract: Polydatin, a natural precursor of resveratrol, has been shown to possess strong antioxidative bioactivity. Recent evidence has suggested that polydatin protects against chronic alcohol exposure and acute hemorrhagic shock in a rodent model of several neurodegenerative disorders, but the mechanisms underlying this protection are largely uncharacterized in Alzheimer's disease (AD). In this study, we determined the neuroprotective effects of polydatin against amyloid beta$_{25-35}$ (Aβ_{25-35})-induced apoptotic cell death in neuronal cells and cognition impairment, and investigated the possible mechanisms involved therein. Polydatin significantly prevented the loss of cell viability, release of lactate dehydrogenase (LDH), accumulation of reactive oxygen species (ROS), and dysfunction of mitochondria membrane potential (MMP) associated with Aβ_{25-35}-induced neurotoxicity in neuronal cells. Polydatin also strikingly inhibited Aβ_{25-35}-induced mitochondrial dysfunction in mice. Moreover, polydatin significantly attenuated Aβ_{25-35}-induced phosphorylation of the c-Jun N-terminal kinase (JNK), p38, and extracellular signal-regulated kinase 1/2 (ERK 1/2) MAPKs, Akt, and glycogen synthase kinase-3 beta (GSK-3β). Furthermore, polydatin significantly inhibited cognition deficits induced by Aβ_{25-35} in mice. Taken together, our results demonstrated that polydatin could protect against neuronal cell death and cognition deficits mediated by Aβ_{25-35}-induced apoptosis via inhibition of the oxidative stress signaling pathway, providing a potential therapeutic agent for the treatment of AD.

Disclosures: **Y. Ko:** None. **S. Lee:** None. **C. Jang:** None.

Poster

600. Alzheimer's Disease: In Vitro Therapeutics

Location: Halls B-H

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Program#/Poster#: 600.10/Q12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HI14C1913

HI15C0527

Title: High-throughput screening (HTS) of an FDA drug library for phosphodiesterase 3 inhibition: effects of selected drugs on lysosomal pH and arrested autophagy

Authors: *H. KIM[1], J.-Y. KOH[1,2];
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Abstract: It has been demonstrated that cAMP acidifies lysosomes via the upregulation of lysosomal vATPase. Such changes may help normalize “arrested autophagy” caused by presenilin-1 mutation (Coffey EE et al. 2014. Neurosci). Currently, several phosphodiesterase inhibitors, which increase cAMP levels, are in clinical use. Previously, we have shown that cilostazol, a PDE3 inhibitor used for its antiplatelet effects, reduces Aβ accumulation in cultured pericytes. Hence, in the present study, we screened about 800 FDA-approved drugs (SCREEN-WELL® FDA v. 2.0 Approved Drug Library, purchased from ENZO) to see if we can find PDE3 inhibitor activities among these drugs. Drug effects on PDE3 activity were measured using PDELight™ HTS cAMP Phosphodiesterase Assay (LONZA). In the presence of PDE3, cAMP turns into AMP and then to ATP, which along with luciferase produces photons. Hence the luminescence reflects the PDE3 activity. Addition of a PDE3 inhibitor would result in the reduction of the luminescence. Add 10 µl of 40 µM inhibitor to each well in compound solvent i.e. 10%(v/v) DMSO. For control wells use compound solvent only. To all wells add 10 µl of PDE3 diluted in reaction buffer at 4x optimal concentration. To all wells add 20 µl of cAMP at 2x optimal concentration. Incubate for 60 minutes at room temperature. Add 10 µl of Stop solution and 20 µl of AMP Detection reagent. Incubate for at least 10 minutes at room temperature. Measure luminescence using a 0.1 second integration time. Using this method, 800 drugs were screened. After two runs (Z’ factors: 0.91 and 0.93 respectively), 16 drugs were found as possible PDE3 inhibitors. Of these, we selected 6, and with individual assays, confirmed that they inhibited PDE3. Like cilostazol, a standard PDE3 inhibitor, these drugs re-acidified lysosomes to variable extents, and increased autophagy flux in a chloroquine model of arrested autophagy in RPE cells. Further experiments in APP/PS-1 expressing CHO cells are under way. Designing new chemicals and developing them into new drugs are a very time-, money- and labor-consuming process. In contrast, finding new indications for pre-existing drug

(drug repositioning) may have much higher chance of success. Since PDE3 inhibitors may help boost lysosomal functions that are often impaired in diverse neurodegenerative conditions, these new indications may prove useful for the treatment of these conditions.

Disclosures: H. Kim: None. **J. Koh:** None.

Poster

600. Alzheimer's Disease: In Vitro Therapeutics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 600.11/Q13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: COLCIENCIAS

DIB-Universisdad Nacional de Colombia.

Title: Liver X receptor agonist GW3965 regulates synaptic function upon amyloid beta exposure in hippocampal neurons

Authors: C. BAEZ[1], A. SANDOVAL[1], F. FILIPELLO[2], H. ARBOLEDA[1], H. MORENO[3], M. MATTEOLI[2], *G. ARBOLEDA[4];
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Abstract: Introducction: Alzheimer`s disease (AD) is characterized by beta-amyloid (Aβ) accumulation and neurofibrillary tangles that are associated to synaptic deficit and dementia. Liver X receptor (LXR) agonist have demonstrated to have beneficial effect in murine models of AD through regulation of Apolipoprotein E, ABCD1, neuroinflamation and Aβ levels. However, the role of LXR in the regulation of synaptic function remains unknown. Aim: To analyzed the in vitro the effect of LXR agonist (GW3965) on the synaptic function upon treatment of hippocampal primary cultures or slices with Aβ. Materials and methods: We used primary hippocampal cultures (DIV 15) or hippocampal slices from C57BL/6 mice; cultures were pre-treated with LXR agonist (GW3965, 1 μM) for 18 hours following by exposure to Aβ (200nM) for 6 hours; expression of synaptic proteins (SV2A, P65 PSD95, shank2, NMDA NR1 y AMPA, GLUA2) was analysed by western blotting; dendritic spines and synaptic contacts were analysed by confocal microscopy by using transfection with a GFP plasmid and by immunocytochemistry for Vglut1 and Shank1, respectively; LTP analysis was performed in hippocampal slices by using a 100 pulses of 100 Hz stimulation protocol. Results: Previously we have demonstrated

that LXR agonist treatment of the triple transgenic mouse model of AD rescued the cognitive decline. Tretament with Aβ induced an increased in the expression level of pre and post-synaptic proteins, a decreased in the number of dendritic spines and synaptic contacts and reduces LTP. LXR agonist reverts all these changes, including an increased in the number of dendritic spines and synaptic contacts, regulation of the expression of synaptic proteins and restores LTP. Conclusion: Our results demonstrate that LXR agonist have an impact in the regulation of synaptic function through modulation of expression of synaptic proteins and synaptic plasticity. LXR agonist may be a pharmacological alternative for AD. Acknowledgements: Funded by COLCIENCIAS and DIB-Universisdad Nacional de Colombia.

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Poster

600. Alzheimer's Disease: In Vitro Therapeutics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 600.12/Q14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association Grant 612330

Title: Novel strategies and screening assays for calcium modulating compounds in Alzheimer's disease models

Authors: *A. LITTLEFIELD[1,2], S. RILEY[2], R. HELFRICH[2], C. BRIGGS[2], N. KAPECKI[2], V. BOTTERO[2], J. BUOLAMWINI[2], R. MARR[2], V. BOTTERO[2], G. E. STUTZMANN[2]; [2]Neurosci., [1]Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: Alzheimer's Disease (AD) is a progressive neurodegenerative disease with many complex pathophysiological features. Of particular interest to our lab is the presence of increased Ca^{2+} release from ER-localized ryanodine receptors (RyR). This dysregulation in Ca^{2+} homeostasis in AD accelerates many disease features, including synaptic deficits. Previous studies have used dantrolene, an RyR negative allosteric modulator, as a tool compound and demonstrated that RyR stabilization restores Ca^{2+} homeostasis and synaptic integrity in AD mice. In order to address the off-target effects and poor solubility of dantrolene, our lab has synthesized and screened a number of dantrolene analogs for their ability to modulate RyR mediated Ca2+ release in neurons from AD mice and model cells. Several of these structural analogs demonstrate RyR-regulating activity similar or superior to dantrolene and are potential novel lead compounds which can preserve synaptic structure and function in AD. In addition, we

have generated novel structural cores, distinct from dantrolene, that show RyR-modifying properties in cellular models of AD.
The current study aims to utilize various cells lines to test the RyR-Ca2+ modulating compounds in order to examine their pharmacologic properties *in vitro*. Using various expression vectors, the multiple cell lines are developed for calcium channel expression patterns, particularly of the three ryanodine receptor isoforms, RyR1, RyR2, and RyR3, and their calcium release properties. The RyR2 isoform is selectively upregulated in both human early-stage AD patients and in AD mouse models and is of particular interest. Immunocytochemistry and qRT-PCR are used to confirm calcium channel expression patterns, while calcium imaging and electrophysiological approaches are used to characterize functional properties. Preliminary data using an RyR2 expressing mouse neuroblastoma (N2a) dose response assay have identified several novel compounds as potent and selective RyR2-Ca2+ modulators with activity comparable to or better than dantrolene. Those with attractive *in vitro* ADME properties have been tested in brain slice preparations from AD mouse models using whole cell patch clamp recordings and 2-photon calcium imaging. Incubating hippocampal brain slices in these lead compounds (10μM) resulted in normalized RyR-evoked calcium responses in the AD brains, while having no effects on other plasma membrane ion channels, passive or active membrane properties, or action potential properties. Thus our novel compounds could illustrate a potential treatment for AD by restoring Ca^{2+} homeostasis and synaptic integrity.

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Poster

600. Alzheimer's Disease: In Vitro Therapeutics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 600.13/R1

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Ferroelectric gold-iron oxide nanoparticles for the photo-thermal dissolution of protein aggregates - implications in alzheimer treatment.

Authors: *S. HARIKUMAR[1], M. ISLAM[2], J. NOVERON[2];
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Abstract: Protein aggregates such as beta-amyloid plaques have been implicated in several neurological diseases such as Alzheimer. Developing chemical tools to induce their

disaggregation may have implications for treatment of these conditions. Prior research has shown that photothermal properties of gold nanoparticles (AuNPs) dispense quanta of heat energy that has been applied in the treatment of cancer cells. Similarly, iron-oxide nanoparticles have been used to deliver drugs to an abrasion within the human body. Our research focuses on developing bio-compatible nanoparticles that consist of an iron-oxide core coated with AuNPs which may be further functionalized with peptides to localize on beta-amyloid plaques. These nanoparticles through Rayleigh Scattering may be triggered to dispense quanta of heat energy to break the hydrophobic bonds between the protein agglomerates to dissolve them. We will present the synthesis and characterization of AuNP-Fe_2O_3 nanoparticles and their surfactant derivatives. The electron microscopy and magnetic moment susceptibility data of the nanoparticles will be presented. In-vitro studies to demonstrate the ability of the nanoparticles to dissolve protein aggregates induced by visible light will be discussed.

Disclosures: S. Harikumar: None. **M. Islam:** None. **J. Noveron:** None.

Poster

600. Alzheimer's Disease: In Vitro Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Horngren Family Alzheimer’s Research Fund

Jean Perkins Foundation

Title: A small molecule ligand for TrkB/TrkC neurotrophin receptors inhibits Aβ induced neurodegeneration *In vitro* and prevents synaptic impairment in Alzheimer’s animal model

Authors: *T. YANG[1], K. C. TRAN[1], A. Y. ZENG[1], S. M. MASSA[2,3], F. M. LONGO[1]; [1]Neurol. and Neurology. Sci., Stanford Univ., Stanford, CA; [2]San Francisco Veterans Affairs Med. Ctr., San Francisco, CA; [3]Neurol., Univ. of California San Francisco, San Francisco, CA

Abstract: In Alzheimer’s disease (AD) toxic oligomers comprised of forms of beta-amyloid (Aβ) and tau accumulate. TrkB and TrkC receptors are expressed by essentially all neurons affected in AD including those in the hippocampus and cortex, where they promote neuronal survival, neurite outgrowth and synaptic plasticity. Our laboratories have developed a non-peptide small molecule, BC-2, that binds to and activates both TrkB and TrkC, but not the TrkA or p75 neurotrophin receptors. We hypothesized that BC-2 might have effects on degenerative processes induced by Aβ *in vitro* as well as in APP-L/S mice. In mouse hippocampal neuron

cultures, which display synapse formation, BC-2 (1000nM) prevented Aβ-induced inactivation of TrkB, AKT and PKC, blocked loss of pre- and post-synaptic markers and inhibited tau phosphorylation. In addition, BC-2 prevented Aβ-induced tau missorting into dendrites, and MC-1 and Tau22 immunostaining indicated reductions of Aβ-induced tau conformational changes and tau oligomerization. Consistent with the ability of TrkB and TrkC to regulate RhoA and Rac activation, BC-2 inhibited Aβ-induced activation of RhoA and inactivation of Rac; this was associated with normalization of diminished cofilin phosphorylation and protection of dendritic spines. In APP-L/S mice, which show progressive plaque deposition in cerebral cortex and hippocampus beginning at 3-4 months of age, BC-2 was administered via intraperitoneal injection once daily (5 days per week) for three months beginning at 4-5 months of age. BC-2 activated TrkB and TrkC in the hippocampus along with their downstream signaling pathways, which was associated with: decreased tau phosphorylation and aberrant conformations; protection of hippocampal apical and basal dendrites and their synaptic spines; and mitigation of behavioral deficits. These findings are consistent with the known intersections of Trk signaling and AD degenerative signaling networks, and point to the possibility of developing clinically viable small molecule ligands for TrkB and/or TrkC receptors as a therapeutic approach for AD.

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Poster

600. Alzheimer's Disease: In Vitro Therapeutics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 600.15/R3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Supported by the Robert and Renee Belfer Family Foundation

Title: The identification of small molecule modulators that increase expression of neuronal survival factor nmnat-2

Authors: J. FRANCIS[1], B. ROTH[2], W. STEBBEDS[1], S. SANYAL[2], R. WILLIAMS[1], G. SMITH[1], M. GECK DO[3], P. JONES[3], *W. J. RAY[2];
[1]BioFocus, LTD, Cambridge, United Kingdom; [2]The Neurodegeneration Consortium, Houston, TX; [3]Inst. for Applied Cancer Sci., Houston, TX

Abstract: Nicotinamide mononucleotide (NAD) adenylyl transferase 2 (NMNAT2) is an essential enzyme for neuronal growth, maintenance and survival. Constant anterograde trafficking of highly labile NMNAT2 from cell body to axon is required to maintain neuronal integrity. Knock-down of endogenous NMNAT2 below a threshold level triggers Wallerian-like axonal degeneration in the absence of injury. Conversely, modest overexpression of NMNAT2 offers protective effects on neuronal degeneration in primary neurons and rescues axonal degeneration in *Drosophila* nerve injury models and a mouse model of tauopathy (rTg4510). Collectively these studies suggest that agents that increase NMNAT2 levels in neurons could have therapeutic potential in neurodegenerative diseases. The mechanisms underlying NMNAT2-mediated neuroprotection remain elusive. There is evidence suggesting the protective effects are afforded by its enzymatic activity that leads to an increase in NAD+ levels and it may act as an enzyme-independent chaperone that assists in clearing neurotoxic misfolded protein aggregates. Here we describe an approach to identify small molecules that enhance NMNAT2 stability and demonstrate neuroprotective effects. We performed a high content image-based high-throughput screen using a stable cell line expressing Flag-tagged NMNAT2 (half-life 100 minutes). Several hits were identified that demonstrated a concentration-dependent increase in levels of Flag-NMNAT2 expression 4 h post-treatment. The lead series of compounds had no effect on the turnover of beta-catenin, another protein rapidly eliminated by the proteasome, or on the membrane localization of CD8, a protein that, like NMNAT2, is palmitoylated. Imaging analyses indicates that this compound increases NMNAT2 expression with the same Golgi-localized subcellular pattern as in untreated controls. Thus the regulation of NMNAT2 is not due to non-selective effects on proteasomal degradation, palmitoylation, or trafficking. Furthermore the increase in NMNAT2 occurred in the presence of cycloheximide, ruling out an effect on transcription. As assessed by western blot, the lead compound enhanced NMNAT2 expression levels by 30-40% in NMNAT2 HEK cells. In agreement with published reports, this modest increase in NMNAT2 was sufficient for biological effect; the compound is neuroprotective in a primary rat dorsal root ganglion NGF withdrawal assay, and completely prevented neurite withdrawal induced by the RhoA-activating toxin CNO3 in human embryonic stem cell-derived neurons. Current studies will delineate the mechanism by which this molecule enhances NMNAT2 expression.

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Poster

600. Alzheimer's Disease: In Vitro Therapeutics

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Scholarship 471779/2010-5

Title: Interaction between proteasomal and lysosomal systems can be modulated to reduce $A\beta_{42}$ effects in hippocampal slice cultures

Authors: *K. G. FARIZATTO[1], U. S. IKONNE[2], H. W. ROMINE[1], M. F. DE ALMEIDA[1], M. F. R. FERRARI[3], B. A. BAHR[1];
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Abstract: Intracellular protein clearance decreases with age, thus altering the vital balance between protein synthesis vs. degradation. Accumulating toxic proteins lead to the activation of proteasomal and lysosomal pathways for clearing such species. However, many studies indicate that the two pathways exhibit stress during AD-type protein accumulations. In this study, low concentration Aβ42 was applied to hippocampal slice cultures resulting in reduced proteasome activity (p=0.0196) in correspondence with increased tau phosphorylation and significant loss of synaptophysin. When the slices were treated with the proteasome inhibitor lactacystin, a nearly complete and rapid reduction in proteasome activity was found. Interestingly, during the proteasomal compromise a putative compensatory response by lysosomal enzyme cathepsin B (CatB) was detected, a 50-75% increase in CatB activity. This suggests potential cross-talk between proteasomes and lysosomes as suggested (Pandey et al. 2007, *Nature* 447:860). To further assess the apparent inverse relationship, we used a lysosomal enhancing agent (Z-Phe-Ala-diazomethylketone; PADK) that promotes protein clearance (Butler et al. 2011: *PLoS One* 6:e20501; Bahr et al. 2012: Rejuvenation Res 15:189) to test for proteasomal attenuation. Surprisingly, instead of a decrease PADK appeared to cause a small increase in proteasome activity (control = 98.7±1.7, PADK = 115.3±12.4, N.S.). In slices with Aβ42-mediated proteasomal compromise (34.7±5.2), PADK indeed enhanced the proteasome activity (73.0±10.0, p=0.027), to levels comparable to control slices. Furthermore, PADK also reduced Aβ42-mediated tau phosphorylation by >50%, an event implicated as a consequence of the negative influence on proteasomes and protein clearance efficiency. Such efficiency may involve cross-talk between proteasomes and lysosomes. Together, the results suggest a distinct interaction between proteasomal and lysosomal systems, and they point to potential dual

modulation against protein accumulation pathology linked to Alzheimer's disease and other dementias.

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Poster

600. Alzheimer's Disease: In Vitro Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

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NIH Grant AG053150

Title: Selection of lead tau oligomer inhibitors for *In vivo* studies

Authors: E. J. DAVIDOWITZ, P. K. KRISHNAMURTHY, P. LOPEZ, C. GLUCHOWSKI, *J. G. MOE;
Oligomerix, Inc., New York, NY

Abstract: There is mounting evidence that tau oligomers have a pathological role in Alzheimer's disease (AD) including leading to the impairment of synaptic function and the spread of pathology. We have undertaken a screening approach to discover small molecule inhibitors that prevent tau oligomer formation at the beginning of the aggregation cascade. Competing programs use methods to select compounds inhibiting the formation of tau fibrils or large aggregates, previously thought to be the most toxic tau species. We hypothesized that by targeting the first step in tau self-association all forms of tau aggregates should be reduced. This work is also highly differentiated from other approaches in that we used full length tau without any mutations that are not relevant to AD. These assays were used to screen a large compound library optimized for drug-like properties. Leads from three chemical series with in vitro IC50 ranging from 300 - 600 nM, molecular weights under 450 Daltons, cLogP 2 - 5, and a polar surface area ranging from 45 to 70 $Å^2$, and which were not toxic to SH-SY5Y neuroblastoma cells, have been selected for further testing of CaCo2 permeability and stability, as well as pharmacokinetic analysis. The best performing candidate compound will be tested in vivo using htau mice. Mice will be treated at 3 escalating doses for 4 months and their brains will be used for biochemical and histological analysis to assess changes in tau oligomer load and efficacy of our treatment paradigm.

Disclosures: **E.J. Davidowitz:** A. Employment/Salary (full or part-time): Oligomerix, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc. **P.K. Krishnamurthy:** A. Employment/Salary (full or part-time): Oligomerix, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc. **P. Lopez:** A. Employment/Salary (full or part-time): Oligomerix, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc. **C. Gluchowski:** A. Employment/Salary (full or part-time): LifeScience Innovations LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); LifeScience Innovations LLC. **J.G. Moe:** A. Employment/Salary (full or part-time): Oligomerix, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc..

Poster

600. Alzheimer's Disease: In Vitro Therapeutics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 600.18/R6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA/NIH SBIR Grant 1R43AG044924-01A1

Kentucky Cabinet for Economic Development, Grant KSTC-184-512-14-181

Title: Development of Glycosaminoglycan-Interacting Small Molecule (GISMO) compounds for the treatment of Alzheimer's disease

Authors: *P. GREGOR[1], R. BERNAT[1], E. CAMPBELL[1], M. P. MURPHY[2], N. HARRIS[4], R. ZHUK[1], B. F. O'HARA[3], M. FINDEIS[1], J. LIU[1], M. FOLEY[1];
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Abstract: The aim of our program is to develop a novel, disease-modifying, orally active therapeutic agent for Alzheimer’s Disease. According to our hypothesis, amyloid-beta (Abeta) is necessary but not sufficient to cause Alzheimer's Disease alone, and the interaction between Abeta and glycosaminoglycans (GAGs) is an essential process leading to the sequence of events resulting in Alzheimer's Disease. As a result, Abeta interaction with GAGs leads to endo-lysosomal accumulation of GAGs (i.e., Abeta/GAG complex), lysosomal storage and

indigestibility of GAGs, which subsequently leads to lysosomal dysfunction and nerve cell death, ultimately contributing to the development of Alzheimer's Disease.
We have discovered a new class of planar aromatic amine compounds (Harris et al. (2014) Biochim. Biophys. Acta, 1840(1):245), Glycosaminoglycan-Interacting Small Molecules (GISMOs), which uniquely targets GAGs, particularly the heparan sulfate GAGs (HS-GAGs). We now report identification of GISMOs that inhibit interactions between amyloid-beta (Abeta) and HS-GAGs, while having potent biological activity in at least two assays relevant to amyloidosis in nerve cells. A set of Abeta/HS-GAG inhibitors, identified by screening in a molecular interaction assay, were tested for inhibition of Abeta42 and Abeta40 uptake into neuronal SH-SY5Y cells, and a number of inhibitors (>30% Abeta uptake inhibition) were identified. Subsequently, 15 compounds were chosen for neuroprotection studies in primary rat brain neuronal cultures, against toxic Abeta42 and Abeta40. Five lead compounds displayed very good efficacy (>50% reversal of Abeta toxic effects at concentrations as low as 0.1 μM), several of which displayed complete reversal of Abeta40-induced LDH release, and Abeta40 and Abeta42-induced caspase 3/7 activation. The five lead compounds have acceptable in vitro safety (cytotoxicity), selectivity, and other drug-like properties. These lead compounds now enter in vivo validation in animal models of Alzheimer's Disease.
This is the first report of Abeta-HS-GAG inhibitors having significant efficacy in neuroprotection against Abeta40 and Abeta42. These results provide in vitro validation for Abeta/HS-GAG interaction as a drug target as well as justification for further development of GISMO compounds as Alzheimer's Disease therapeutics. GISMOs may also have therapeutic potential in other amyloid neurodegenerative diseases associated with amyloid peptides, i.e., Parkinson's Disease (α-synuclein), Frontotemporal Dementia and other tauopathies (tau) and Amyotrophic Lateral Sclerosis (TDP-43, SOD1).

Disclosures: **P. Gregor:** None. **R. Bernat:** None. **E. Campbell:** None. **M.P. Murphy:** None. **N. Harris:** None. **R. Zhuk:** None. **B.F. O'Hara:** None. **M. Findeis:** None. **J. Liu:** None. **M. Foley:** None.

Poster

600. Alzheimer's Disease: In Vitro Therapeutics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 600.19/R7

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The mechanism of axonal transport deficit caused by downregulation of O-GlcNAcylation in Alzheimer's disease

Authors: *H. KIM, H. CHOI, W. LEE, I. MOOK-JUNG;
Seoul Natl. Univ. (college of Medicine), Seoul-City, Korea, Republic of

Abstract: Glycosylation with O-linked β-N-acetylglucosamine (O-GlcNAc) is one of the protein post-translational modification that regulates various protein activities. In Alzheimer's disease (AD), protein O-GlcNAc level is downregulated. This downregulation causes harmful effects on cells, especially neuron. Also, axonal transport deficit occurs in AD. This is up to dysfunction of motor and adaptor proteins. Here, we verified that Aβ induced axonal transport deficit was recovered by O-GlcNAcase inhibitor (Thiamet-G). Also the axonal transport related protein altered O-GlcNAc level in Aβ treated cells, as well as in transgenic mouse model were targeted. Site mutation was constructed to silence O-GlcNAcylation on the protein. This construct was used for discovering mechanisms of between O-GlcNAcylation downregulation and axonal transport deficit in AD. This study discover relationship with O-GlcNAc and axonal transport in AD, and it is expected to be support development of specific therapeutic strategy.

Disclosures: H. Kim: None. **H. Choi:** None. **W. Lee:** None. **I. Mook-Jung:** None.

Poster

600. Alzheimer's Disease: In Vitro Therapeutics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 600.20/R8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Swedish Brain Foundation

Marianne and Marcus Wallenberg Foundation

Cure Alzheimer's

Title: A CRISPR-Cas9 based strategy to target the Swedish APP allele in a familial form of Alzheimer's disease

Authors: *C. I. LOOV[1], B. GYORGY[1,2], S. TAKEDA[1], C. COMMINS[1], M. ZABOROWSKI[1], D. MU[1], A. VOLAK[1], L. LANNFELT[3], C. A. MAGUIRE[1], B. T. HYMAN[1], X. O. BREAKEFIELD[1], M. INGELSSON[1,3];
[1]Neurol., Massachusetts Gen. Hosp., Charlestown, MA; [2]Howard Hughes Med. Inst., Boston, MA; [3]Dept. of Publ. Health, Mol. Geriatrics, Uppsala Univ., Uppsala, Sweden

Abstract: Mutations in the *amyloid precursor protein* (*APP*) gene cause early-onset forms of Alzheimer's disease. The *APPswe* mutation leads to an increased beta-secretase cleavage of

APP, resulting in higher levels of amyloid-β (Aβ) 40 and Aβ42 in both brain and peripheral tissues, such as fibroblasts. The development of gene editing tools such as the clustered regularly interspaced short palindromic repeats (CRISPR) system offers novel possibilities to selectively target and disrupt alleles with disease-causing mutations through double-stranded breaks and mismatch repair. In this study we aimed to use the CRIPSR-Cas9 system to target *APPswe* in order to reduce Aβ levels. Three different *APPswe*-directed and one *APPwt*-directed gRNAs were designed. Each gRNA was tested individually together with the Cas9 linked to green fluorescent protein (GFP) into fibroblasts from two mutation carriers and two non-mutation carriers. Transfected (GFP+) cells were separated from non-transfected (GFP-) cells by FACS and replated for additional culturing. Upon confluency, the cells were cultured for 48h in serum-free media before the media was saved for Aβ analysis and cells collected for DNA extraction. Sanger and next generation sequencing showed that the respective gRNAs had varying degrees of specificity against the *APPswe* and *APPwt* alleles, respectively. The longest *APPswe* gRNA (SW1) showed the highest efficacy and almost completely eliminated the mutated allele (99.92%) while conserving the wt allele. Moreover, the media from the fibroblasts showed the predicted decrease of both Aβ40 and Aβ42, as measured by ELISA. Next, the SW1 *APPswe* gRNA and Cas9 were packaged into separate adeno-associated viruses that were used to co-transduced primary neuronal cultures from embryos of the Tg2576 *APP* mouse model. Although these transgenic mice carry multiple copies of the *APPswe* gene, next generation sequencing showed that approximately 3-4% of the *APPswe* alleles were disrupted in the transduced neurons. Taken together, our data indicate that the CRISPR-Cas 9 system can be used to selectively target an *APP* mutation causing Alzheimer's disease and efficiently decrease the production of Aβ in cells carrying such a mutation.

Disclosures: **C.I. Loov:** None. **B. Gyorgy:** None. **S. Takeda:** None. **C. Commins:** None. **M. Zaborowski:** None. **D. Mu:** None. **A. Volak:** None. **L. Lannfelt:** None. **C.A. Maguire:** None. **B.T. Hyman:** None. **X.O. Breakefield:** None. **M. Ingelsson:** None.

Poster

600. Alzheimer's Disease: In Vitro Therapeutics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 600.21/R9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R43 AG043203

R44 NS086343-01

Title: Novel tricyclic pyrone compounds cp2 and tp70 differentially modulate glutamatmergic neurotransmission and synaptic plasticity in the rat hippocampus

Authors: *B. ZOU[1], W. CAO[1], C. PASCUAL[1], K. XIAO[1], S. WEERASEKARA[2], M. ZHANG[2], I. MAEZAWA[3], L.-W. JIN[3], D. HUA[2], X. XIE[1];
[1]AfaSci Res. Labs., Redwood City, CA; [2]Dept. of Chem., Kansas State Univ., Manhattan, KS; [3]Dept. of Pathology and Lab. Med., Univ. of California Davis, Sacramento, CA

Abstract: Alzheimer's disease is associated with abnormalities in several neurotransmitter pathways, especially the glutamatergic system. Overactivation of glutamate receptors, particularly NMDA, may contribute to neurodegeneration. Memantine, an activity-dependent NMDA receptor antagonist with moderate-affinity, produces neuroprotection and has been used for the treatment of Alzheimer's disease. However, in the late stage of the disease, the NMDA receptor system is hypoactive and stimulation of the NMDA system may mitigate the cognitive decline. In the present study, we have found that the novel tricyclic pyrone compounds CP2 and TP70 with similar structures differentially modulated the glutamatergic system. Using extracellular recordings of rat hippocampal brain slices, CP2 (1-10μM) inhibited the basal field EPSP, blocked the induction and maintenance of long-term potentiation (LTP) induced by high frequency stimulation. Both CP2 and memantine (10μM) comparably inhibited the NMDA receptor-mediated EPSP in a reversible and activity-dependent manner. In contrast, TP70 (1-10μM) caused a long-lasting enhancement of the NMDA receptor-mediated EPSP (133.6±9.5% of control in 10μM) and increased the magnitude of NMDA-dependent LTP (246.7±20.7% vs 191.4±17.5% in control) with little effects on basal EPSP predominantly mediated by AMPA receptors. Furthermore TP70 restored the Aß-42 oligomer-impaired LTP. Aß-42 oligomer (0.1 μM) alone decreased the magnitude of LTP (145.3±10.9%), while co-application with TP70, LTP (180.1±17.5%) was maintained towards the control level. Interestingly, despite the differential modulation on the excitatory neurotransmission and synaptic plasticity, CP2 and TP70 each (25mg/kg, p.o. over 3 months of treatment) reduced cerebral amyloid load by approximately 30% in Alzheimer's disease transgenic mouse brains of both 5xFAD and APP-PS1 revealed by histopathological studies. These results suggest that CP2 may protect against excitotoxicity and may potentially be developed as a therapeutic targeting glutamatergic hyperactivity to decelerate neurodegeneration. TP70 can stimulate the NMDA receptor system and may form the basis for the development of a potential therapy to alleviate the loss of memory associated with the dysfunctional glutamatergic system in Alzheimer's disease.

Disclosures: B. Zou: None. **W. Cao:** None. **C. pascual:** None. **K. Xiao:** None. **S. Weerasekara:** None. **M. Zhang:** None. **I. Maezawa:** None. **L. Jin:** None. **D. Hua:** None. **X. Xie:** None.

Poster

600. Alzheimer's Disease: In Vitro Therapeutics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 600.22/R10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Rosetrees Trust

Title: Neuroprotective effets of Liraglutide against chronic endoplasmic reticulum-stress

Authors: *T. PANAGAKI, C. HOLSCHER;
Biomed. and Life Sci., Lancaster Univ., Lancaster, United Kingdom

Abstract: Alzheimer's disease (AD) has been emerging as the most prevalent and socially disrupting malady of the elderly, with more than 44 million cases worldwide currently developing dementia. The molecular determinants underlying the disease onset and progression remain poorly understood while existing treatments solely provide a short-lived symptomatic relief. Improved understandings of AD etiopathogenesis along with the discovery of disease-modifying drugs are now the pharmacologic research priorities. Accumulating evidence suggests that the persistent endoplasmic reticulum (ER) stress may drive impairments in neuronal integrity and function. The ER stress engages a homeostatic signaling network that orchestrates various processes - from protein quality control and energy homeostasis to inflammation and cell differentiation - to control organelle homeostasis and cell fate. Pharmacological or genetic attenuation of ER stress has successfully restored pathological features and behavioral impairments of diverse animal models of neurodegenerative disorders, rendering ER stress a potent target for therapeutic intervention. Here, we examine whether the glucagon-like peptide 1 (GLP1) analogue Liraglutide can combat the persistent ER stress in the neuroblastoma SH-SY5Y cell line. Chronic treatment with thapsigargin, a non-competitive inhibitor of sarco/ER Ca^{2+}-ATPase, significantly reduced cell proliferation and impaired 5-bromo-2'-deoxyuridine (BrdU) incorporation and 2,3-Bis-(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide (XTT) metabolism *in vitro,* accompanied by an abnormal increase in lactate dehydrogenase (LDH) levels in the culture supernatant, indicating increased cell death. The impairments in cell physiology further correlate with an increase in the expression levels of the ER luminal stress protein BiP, the apoptosis-related transcription factor Chop and the ER resident caspase 12 (CASP12). Liraglutide co-treatment alleviates the ER stress-induced impairments in cell proliferation and viability and abnormal rise in cytotoxicity. It further attenuates the upregulated expression of the ER stress markers BiP, Chop, and CASP12. To conclude, persistent ER stress detrimentally affects neuronal cell fate, which can be prevented by Liraglutide. Liraglutide is currently in clinical trials in Alzheimer’s patients, and the results presented here and previously demonstrate that the drug shows promise to improve disease progression.

Disclosures: **T. Panagaki:** None. **C. Holscher:** None.

Poster

600. Alzheimer's Disease: In Vitro Therapeutics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 600.23/R11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Wellcome Trust Seed Grant

Title: Design and development of a small molecule therapeutic targeting both beta-amyloid and tau misfolding

Authors: ***D. F. WEAVER**[1], S. BANFIELD[2], C. BARDEN[1], A. BHATTACHARYA[2], K. KESKAR[1], E. LU[2], M. REED[2], B. SWEETING[2], M. TAYLOR[2], Y. WANG[2], F. WU[2], A. YADAV[2], S.-P. YANG[2];
[1]Krembil Res. Institute, UHN, Toronto, ON, Canada; [2]Treventis Corp., Toronto, ON, Canada

Abstract: Clinically, Alzheimer's disease (AD) manifests as progressive deterioration in memory and cognition; pathologically, these symptoms arise from cytotoxic protein misfolding, primarily oligomerization of beta-amyloid (Abeta) and tau. To rationally discover a disease modifying drug for AD via exploitation of a druggable target, we devised an in silico 3D molecular model designated as CCM (Common Conformational Morphology), where CCM is the abnormal protein shape common to both beta-amyloid and tau that predisposes to their proteopathic misfolding. Next, a library of 11.8 million compounds was filtered for CNS properties and screened in silico against CCM leading to 3,082 hits. Based on these hits, more than 620 prototype compounds were synthesized, culminating in the identification of the TRV200 class of compounds, which underwent extensive analogue synthesis (>500 analogues) to enable structure-activity optimization. The TRV200 class consists of novel, brain-penetrant small molecules capable of preventing the sporadic or templated misfolding of monomeric proteins, thereby blocking the generation of neurotoxic and synaptotoxic transient oligomeric species (OAbeta, Otau). Single compounds within the TRV200 class block oligomerization of both Abeta and tau (IC50 < 1 μM) in a dose-dependent manner as exhibited in vitro using biotin-amyloid and biotin-tau assays. In surface plasmon resonance studies, TRV200 compounds show selective binding to monomeric Abeta. Compounds show attenuation of tau misfolding by mass spec techniques and are also active in cell-based tau aggregation assays. Compounds exhibit in vivo brain bioavailability, engaging/reducing both Abeta and tau targets in transgenic mouse models (APP-PS1, Tg4510); e.g., a TRV200 class compound decreases Abeta oligomer concentrations within interstitial fluid by 40% within 72 hrs of administration and tau oligomer

concentrations by 35% within 7 days of oral dosing. These compounds also rescue both synaptic function and behavioural deficits in APP-PS1 mice in long-term potentiation, radial arm water maze and fear conditioning testing, following 8 weeks of administration (30 mg/kg po od n=13). Lead compounds from the TRV200 class demonstrate favourable drug metabolism-pharmacokinetic (DMPK) properties such as high liver microsome stability and low CYP inhibition and induction. The compounds are non-genotoxic and show low off-target promiscuity (receptor profiling, protein-protein inhibition, hERG). Thus, we have developed a new class of drug-like, orally bioavailable, brain-penetrant small molecules capable of simultaneously inhibiting both Abeta and tau oligomerization.

Disclosures: **D.F. Weaver:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Treventis Corp. **S. Banfield:** A. Employment/Salary (full or part-time): Treventis Corp. **C. Barden:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Treventis Corp. **A. Bhattacharya:** A. Employment/Salary (full or part-time): Treventis Corp.. **K. Keskar:** None. **E. Lu:** A. Employment/Salary (full or part-time): Treventis Corp. **M. Reed:** A. Employment/Salary (full or part-time): Treventis Corp. **B. Sweeting:** A. Employment/Salary (full or part-time): Treventis Corp. **M. Taylor:** A. Employment/Salary (full or part-time): Treventis Corp. **Y. Wang:** A. Employment/Salary (full or part-time): Treventis Corp. **F. Wu:** A. Employment/Salary (full or part-time): Treventis Corp. **A. Yadav:** A. Employment/Salary (full or part-time): Treventis Corp. **S. Yang:** A. Employment/Salary (full or part-time): Treventis Corp..

Poster

600. Alzheimer's Disease: In Vitro Therapeutics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 600.24/R12

Topic: C.01. Brain Wellness and Aging

Support: University Start-up Funding #R9321

NIH (NS71022)

Title: P53-mediated senescent neurons trigger apoptosis of healthy bystanders by modulating the metabolic microenvironment in the brain

Authors: ***H. CHOW**[1,2], K. HERRUP[1];
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Abstract: Aging is the greatest risk factor for AD yet its molecular mechanisms are not well understood. One clue we have been pursuing is that excessive cellular senescence correlates with premature aging phenotypes. For example, both neurofibrillary tangles and neuritic components of the plaques of AD patients show strong immunoreactivity for markers of senescence such as p16(INK4A). Our preliminary findings in human AD and related mouse materials revealed substantial number of neurons show signs of p53-mediated senescence triggered by aberrant p25/β-catenin stabilization. These senescent neurons have lost significant number of neurites, but are functionally "undead" and in this state they are capable of triggering apoptosis in nearby, otherwise healthy, neurons. An additional major correlate of the aging process is altered metabolic regulation. Here again there is a p53 connection as sustained stabilization of p53 impedes the usage of glucose by suppressing glycolysis, and at the same time enhances the usage of glutamine thus sustaining TCA cycle intermediates and glutathione synthesis in senescent neurons. These changes reshape the metabolic microenvironment, depleting the availability of glutamine for the bystander neurons, and rendering them hypersensitive to different types of stress. Further, pharmacological inhibition of p53, or supplementation of glutamine, prevented the bystander killing triggered by senescent neurons. Together, our observations suggest that p53-stabilization induces neuronal senescence and bystander apoptosis by modulating the metabolic microenvironment in the brain. The larger implication of these findings is that pharmacological inhibition of nuclear p53 activities and glutamine supplementation may benefit to the treatment of AD.

Disclosures: H. Chow: None. **K. Herrup:** None.

Poster

600. Alzheimer's Disease: In Vitro Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 1R01AG042890 (GT)

Mitchell Center for Neurodegenerative Diseases

T32ES007254 (OZ)

Title: Epigenetic modulation of synaptic resistance to amyloid-beta

Authors: *O. ZOLOCHEVSKA[1], R. WOLTJER[2], G. TAGLIALATELA[1];
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Abstract: Alzheimer's Disease (AD), the sixth leading cause of death in the US, is the most common form of age-associated dementia, accompanied by synaptic loss at early stages, and neuronal death at late stages of the disease. Oligomeric aggregates are considered to be the most toxic species of the two hallmark misfolded proteins in AD, amyloid beta (Abeta) and tau, capable of targeting and disrupting synapse and thus driving cognitive decay. However, certain individuals (here referred to as Non-Demented with Alzheimer's Neuropathology - NDAN) are capable to withstand the toxicity caused by Abeta and tau, and thus preserve cognitive competency despite the presence of AD neuropathology. Understanding the involved mechanism(s) would reveal new, effective treatment targets. We have previously shown that the post-synaptic density (PSD) of NDAN subjects is capable of rejecting Abeta, thus shielding synapses from its toxicity and, therefore, preserving cognitive ability. We have now completed the proteomics analysis of PSD fractions of hippocampi of control, AD and NDAN subjects and found that 31 unique proteins are significantly different between AD and NDAN PSDs. Ingenuity pathway analysis (IPA) was then performed to determine the canonical pathways, interactions among networks and upstream regulators. Several microRNAs were identified by IPA as potential drivers of the changes we have observed at the PSDs. Among these, we confirmed that microRNA-485, -4723 and -149 are differentially expressed in AD vs control and NDAN brains. Specifically, microRNA-485 and -149 are upregulated in AD, while microRNA-4723 is inhibited. Therefore, we hypothesize that changes in microRNA have an important role in either synapse protection or sensitization to Abeta binding. In order to test our hypothesis, we used a cellular model (differentiated SH-SY5Y cells) to determine if modulation of such microRNA had an effect on the ability of Abeta to associate with synaptic elements. SH-SY5Y cells were transfected with miRNA-4723 mimic and microRNA-485 inhibitor, and then treated with HiLyte Fluor 647-labeled Abeta oligomers. Abeta binding was evaluated using flow cytometry. Taken together, our findings suggest that there is a unique regulation of microRNA in NDAN which could be responsible for protection of synapses from Abeta toxicity, thus contributing to retention of cognitive ability.

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Poster

600. Alzheimer's Disease: In Vitro Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH awards AG043415, AG031311, NS056051 and MH096972

Thome Memorial Foundation Award,

ADDF Award 261108

Simons Foundation Autism Research Initiative

Title: A discovery platform for selective and efficacious CNS drug candidates with high safety potential: a stress protein kinase inhibitor case study for potential disease modification.

Authors: *D. WATTERSON[1], S. M. ROY[1], J. SCHAVOCKY[1], V. GRUM-TOKARS[1], A. BACHSTETTER[3], G. MINASOV[2], M. J. ROBSON[4], W. ANDERSON[2], R. BLAKELY[4], L. VAN ELDIK[3], J. PELLETIER[1], O. ARANCIO[5];
[1]Dept. of Pharmacol., [2]Dept. of Biochem., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; [3]Univ. of Kentucky, Lexington, KY; [4]Florida Atlantic Univ., Jupiter, FL; [5]Columbia Univ., New York, NY

Abstract: A key aspect of our discovery platform for novel in vivo probes and drug candidates is the use of informatics and curated databases of small molecules with in vivo CNS data in animals or humans as well as in vitro molecular property profiling. The limited number of small molecules falling within this in vivo relevant molecular space provides a filter for initial synthetic chemistry efforts. Pharmacological screens on initial compounds provide an additional, project-specific filter for recursive medicinal chemistry refinement. The decision tree terminates non-essential synthetic efforts and focuses on optimization in single target and functional discovery. Functional approach deliverables are now in the investigational new drug (IND) enabling or early clinical stage for Alzheimer's (AD) and brain injury. The case study presented here is for the established but technically challenging p38aMAPK target. Briefly, we exploited the CNS scaffold aminoarylpyridazine, a viable molecular fragment (PDB 4ZTH). Our experimental approach used high-resolution crystallography of small molecule-kinase complexes and safety pharmacology screens. The first compound synthesized and tested, MW069a (PDB 4EWQ), had a promising in vitro and in vivo profile but lacked the desired kinase specificity and safety pharmacology potential. Med chem refinement delivered second generation in vivo probes (MW108, PDB 4F9W ; MW181, PDB 4F9Y) with the desired isoform selectivity and improved safety potential. At this stage we also needed to use the platform to address and revise prevailing dogma about active site targeting of PK inhibitors. Pharmacokinetic optimization and safety pharmacology drove the final stage of med chem optimization that yielded MW150 (PDB 4R3C). MW150 is an isoform selective p38aMAPK inhibitor candidate with excellent in vivo exposure and safety in preclinical drug development. A regulatory compatible drug production scheme is available. Efficacy in AD models and an autism susceptibility model, combined with target engagement and pharmacodynamic marker analyses, document the MW150 potential as a candidate for development. The results address the translational gap between the knowledge that PKs are critical players in CNS disease progression and the unmet need for highly selective and CNS-active PK inhibitors that are viable candidates for future clinical drug development.

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Poster

600. Alzheimer's Disease: In Vitro Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R21-AG048506

NIH Grant P01-AG000538

NIH Grant RO1-AG34667

Title: Enhancement of cGMP signaling rescues LTP in Alzheimer diseased synapses: a high throughput analysis

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Abstract: Maintaining and even improving cognitive function in early Alzheimer' disease (AD) is a major goal of modern medicine. However, no effective treatments for cognitive decline currently exist. Because it is generally accepted that cognitive decline is due to synaptic dysfunction, the initial evaluation of therapeutics is commonly based on the analysis of long-term potentiation (LTP), a cellular correlate of learning and memory. Phosphodiesterases inhibitors (PDEi), which prevent the breakdown of second messengers cAMP and cGMP, represent a potential treatment strategy for memory decline and have been recognized as therapeutic targets for cognitive improvement and AD. Here we use our novel method to track chemically-induced LTP in isolated synaptosomes ('*Fluorescence Analysis of Single-Synapse Long-Term Potentiation*', FASS-LTP) for drug evaluation in cryopreserved brain samples from AD cases. We simultaneously tested 7 selective PDEi. In parallel, we tested forskolin (adenylyl cyclase activator, AC_{activ}) and a novel guanylyl cyclase activator (GC_{activ}), as well as functionally relevant combinations of PDEi with AC_{activ} or GC_{activ}. Thus, a total of 18 treatments were simultaneously tested on basal conditions and after cLTP. We found that, when combined with GC_{activ}, inhibition of cGMP-specific PDEi rescued cLTP in synaptosomes from AD cases ($P < 0.05$). Basal levels of potentiated synapses were not affected by any drug treatment. These data

show that FASS-LTP is a sensitive and efficient approach for drug screening in human synapses, a previously unattainable goal. Notably, a molecular target were discovered to be the cGMP signaling pathway, suggesting that synaptic plasticity in the AD brain may be rescued by cGMP-specific PDEi.

Disclosures: **G.A. Prieto:** None. **C.T. Dang:** None. **C.W. Cotman:** None.

Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 601.01/R16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSFC Grant 81273489

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Title: Blocking the interaction between EphB2 and ADDLs by a small peptide rescues impaired synaptic plasticity and memory deficits in a mouse model of Alzheimer’s disease

Authors: ***C. GAO**, X.-D. SHI, K. SUN, R. HU, N. SUN, J.-R. HAO, Y. HAN, L.-C. ZHANG; Xuzhou Med. Col., Jiangsu, China

Abstract: Soluble amyloid-beta (Aβ) oligomers, also known as Aβ-derived diffusible ligands (ADDLs), are thought to be the key pathogenic factor in Alzheimer's disease (AD). But there is still no effective treatment for preventing or reversing the progression of the disease. Targeting N-methyl-D-aspartic acid (NMDA) receptors trafficking and its regulation is a new strategy for AD early treatment. Aβ oligomers have been found to bind to the fibronectin type III repeats (FN) domain of EphB2 to trigger EphB2 degradation, thereby impairing the normal functioning of NMDA receptors and resulting in cognitive deficits. Here, we identified for the first time the

interaction sites of the EphB2 FN domain with ADDLs by applying peptide array method, based on which to design and synthesize four candidate peptides (Pep21, Pep25, Pep32 and Pep63) that might be able to block EphB2-ADDLs interaction. Among them, Pep63 was screened out to be the most effectively one to inhibit the binding between EphB2 and ADDLs. We found that Pep63 not only rescued the ADDLs-induced depletion of EphB2 and GluN2B-containing NMDA receptors from the neuronal surface in cultured hippocampal neurons, but also improved impaired memory deficits in APPswe/PS1dE9 (APP/PS1) transgenic mice as well as the phosphorylation and surface expression of GluN2B-containing NMDA. Together, these results suggest that blocking the EphB2-ADDLs interaction by small interfering peptides may be a promising strategy for AD treatment. **Acknowledgements:** This work was supported by the National Natural Science Foundation of China (Grant No. 81273489, 81471101, and 81300930), the Natural Science Foundation of Jiangsu Province (Grant No. BK2012582 and BK20130232), the Education Department of Jiangsu Province (Grant No. 12KJA180008 and 14KJB320025), and the Qing Lan Project of Jiangsu Province.

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Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 601.02/R17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NRF Grant

Title: Attenuations of learning and memory impairments in aged and transgenic mice models of Alzheimer's disease by mild stress

Authors: C. LEE[1,3], J.-H. JANG[3], *G. PARK[2];
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Abstract: Stress is regarded as one of the critical risk factors for neurodegeneration leading to learning and memory deficits. Although several researchers have reported that mild stress could enhance cognitive functions, its underlying molecular mechanisms are not clearly verified. In this study we have investigated the effect of mild restraint stress (MRS) for 28 days (4h/day) against the learning and memory dysfunction in aged model mice as well as triple transgenic

model mice of Alzheimer's disease (3xTg AD) by conducting diverse behavior tests and molecular analyses. MRS improved mean escape latency, the time taken to find the platform during training trials in Morris water-maze test. In addition, the neuropathological markers for AD such as accumulation of beta-amyloid peptide and hyperphosphorylation of tau protein were mitigated by MRS. MRS effectively decreased ratio of pro-apoptotic Bax to anti-apoptotic Bcl-2, the representative proteins involved in apoptosis. To elucidate the neuroprotective mechanism of MRS, we have examined the molecules involved in the oxidative stress and inflammation. MRS attenuated the lipid peroxidation and protein oxidation through up-regulation of antioxidant enzymes via modulating redox-sensitive proteins such as NF-E2-related factor 2 (Nrf2) and p66(Shc). MRS also attenuated the pro-inflammatory responses by suppressing expression of cytokines such as TNF-α in aged mice. Moreover, MRS increased the expression of brain-derived neurotrophic factor by phosphorylation of cAMP response element-binding protein in 3xTg AD mice. Taken together, these findings suggest that MRS may have beneficial effects for the learning and memory impairments during neurodegenerative process by decreasing neuropathological markers of AD and oxidative stress as well as inflammatory responses, and increasing neurotrophic factors of regeneration.

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Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 601.03/S1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSERC

Weston Brain Institute

Title: Impaired object processing in 3xtg, 5xfad and app/ps1 mouse models of alzheimer's disease: going beyond "object recognition"

Authors: ***S. D. CREIGHTON**[1], D. PALMER[1], V. F. PRADO[2], M. A. M. PRADO[2], B. D. WINTERS[1];
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Abstract: Object recognition evaluates declarative-like memory in rodents and is sensitive to cognitive deficits in transgenic mouse models of Alzheimer's disease (AD). Object recognition, however, is not a unitary process, and there are many uncharacterized facets of object processing that have relevance to AD. To elucidate the specific nature of object processing deficits in transgenic mouse models of AD, we are systematically evaluating performance on tasks that manipulate different types of object information: object identity (ie., object recognition *per se*: OR), spatial processing (object location; OL), temporal processing (temporal order; TO), and multisensory perceptual integration (multisensory object oddity; MSO) in 12-month-old male and female 3xTG, 5xFAD and APP/PS1 mice. Preliminary results are consistent with a multifaceted impairment in object processing. Specifically, 3xTG males were impaired on open-field OR when the retention delay was 5min or 3h, whereas females were selectively impaired at 3h. However, when spatial and contextual cues were minimized, using a modified Y-apparatus, both 3xTG males and females had intact OR at 5min. Conversely, 5xFAD males were impaired on open-field OR at 5min and 3h, but selectively impaired at 3h on Y-apparatus OR, whereas 5xFAD females were impaired on open-field and Y-apparatus OR at 5min and 3h. OL was impaired in 3xTG males, females and 5xFAD females at 5min and 3h. TO was impaired in 3xTG females at 3min. MSO was impaired in 3xTG males, females and 5xFAD females, despite intact basic visual and tactile object perception. Our results reveal dissociations across transgenic AD strains, sex, and type of object information processing, and should ultimately help to clarify the relationship between specific aspects of AD pathology and "object recognition" impairment.

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Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

Location: Halls B-H

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Program#/Poster#: 601.04/S2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Seed Funding for Basic Research 201311159069, HKU

Seed Funding for Basic Research 201311159171, HKU

Title: The effect of varenicline on cognitive dysfunction induced by laparotomy in aged mice

Authors: *C. HUANG[1,2], O. T.-W. NG[2,3], J. M.-T. CHU[2,1], R. C.-C. CHANG[2,4,3], G. T.-C. WONG[1,4];

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Abstract: Postoperative cognitive dysfunction (POCD) occurs more frequently in the elderly and has characteristics in common with other neurodegenerative diseases such as Alzheimer's Disease (AD). Neuroinflammation and abnormal tau protein phosphorylation or mislocation is known to be strongly associated with development of AD. Cholinergic system manipulation has been considered a potential strategy in the treatment of this disease. This study investigates the contribution of surgical trauma to and the effect of the cholinergic agonist varenicline on the development of POCD. Aged male C57BL/6N mice (18-month-old, 40.86 ± 5.54 g) were divided into control (CON), sevoflurane only (SEVO) and laparotomy (LAP) groups, all three groups either treated with vehicle (normal saline) or varenicline, which was administrated orally and daily from one day before treatments continuously for 13 days. Cognitive function was assessed by Novel Objective Recognition test (NOR) and Y-maze analysis, and locomotor activity by the Open Field Test (OFT), motor function by Rotarod test on postoperative day 14. Inflammatory cytokine mRNA expression from the liver, frontal cortex and hippocampus were assessed by q-PCR at 4h postoperatively in three groups. Data were analyzed by One-Way ANOVA followed by Turkey's post hoc test, and $p < 0.05$ was considered as statistically significant. Locomotor activity and motor function were not affected by laparotomy or varenicline with no difference found in the frequency of crossing the square and central duration in OFT, or in latency to fall in Rotarod test. Cognitive dysfunction was observed in LAP group when compared with SEVO group. There was an increase in latency and error number in the Y-maze test, and a lower discrimination index in NOR. However, these changes were reversed by varenicline. Hepatic mRNA levels of IL-1β, TNF-α, IL-6 and MCP-1 were significantly increased in LAP group compared with SEVO group. IL-1β, IL-6 and MCP-1 were significantly elevated in the hippocampus in LAP group. Immunoflurescent intensity of GFAP-positive astrocyte was higher and there was more activated microglia in CA1 and DG regions of the hippocampus in LAP group at 14 d postoperation. Varenicline reduced this high level of immunoreactivity of astrocyte and microglia. There was significant hyperphosphorylation of tau protein (AT8) in both cytosol and nucleus in LAP group. Varenicline decreased tau protein phosphorylation in both regions. Neuroinflammation and tau protein hyperphosphorylation may play important role in the development of cognitive dysfunction induced by laparotomy and varenicline improved cognition by reversing these changes.

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Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 601.05/S3

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Endurance training increases FGF21 expression and improves memory function in Alzheimer's disease mice

Authors: *M. CHU[1,2], G. WONG[2], R. CHANG[1];
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[2]Anaesthesiology, HKU, Hong Kong, China

Abstract: Alzheimer's disease (AD) is one of the most common types of dementia without effective pharmacological treatment. Increasing evidences have shown that physical training could counteract the deterioration of cognitive functions in both human and animal models. However, the underlying mechanism and the responsible molecule for exercise related neuroprotection remain largely unknown. FGF21 is an endogenous metabolic regulator for glucose and lipid which is primarily expressed in liver, muscle, adipose tissues and brain. Recently, FGF21 has been shown to possess important role in mediating insulin signaling activities, neuroprotection and cognition and can be regulated by exercise. In current study, AD animals received 1 month endurance training and the effect of exercise on AD was investigated. Memory function, insulin signaling activities and tau phosphorylation in brain were examined after exercise training. Also, the effect of endurance training on FGF21 gene expression in both peripheral organs and brain were also examined.
For exercise protocol, mice were trained and run on an automatic rotarod instrument for 30 minute per day and 5 times per week. After 1 month training, memory function was examined by novel object recognition test and a modified Y-maze. Insulin signaling activities and tau phosphorylation in frontal cortex were examined by Western blotting. Gene expression of FGF21 in liver, abdominal adipose tissues, soleus muscle and cortex was examined by real time PCR.
After 1 month endurance training, 3x transgenic mice were shown to improve the cognitive performance in novel object recognition test and Y-maze test. Significant improvement of insulin signaling activities and decreased tau phosphorylation were observed in frontal cortex of AD mice after 1 month endurance training. At the same time, mRNA expression of FGF21 in liver, muscle and fat tissues were increased but not in the brain. These results implicated that exercise exerted neuroprotection and induced peripheral FGF21 induction in AD animal model. Further experiments are needed to confirm the role of exercise induced peripheral FGF21 in terms of counteracting AD.

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Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 601.06/S4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH GM060665

NIMHD MD007599

Title: Infusion of the neuroprotective peptide PACAP27 into mouse hippocampi prevents learning and memory deficits elicited by the cyclooxygenase product of inflammation prostaglandin J2

Authors: ***J. A. AVILA**[1,3], M. KIPROWSKA[2,4], T. JEAN-LOUIS[2,4], P. A. SERRANO[1,3], P. ROCKWELL[2,4], M. E. FIGUEIREDO-PEREIRA[2,4];
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Abstract: Upregulation of cyclooxygenase-2 has emerged as an important determinant of the cytotoxicity associated with neuroinflammation in Alzheimer's Disease (AD). Prostaglandins (PGs) are major products of cyclooxygenases, but their role in neurodegeneration is poorly understood. PGD2 is the most abundant prostaglandin in the brain and increases the most under pathological conditions. Specifically, cortical neurons in AD brains were shown to exhibit accelerated PGD2 production. PGD2 is unstable and is converted to the highly neurotoxic prostaglandin J2 (PGJ2) by spontaneous dehydration. To study the *in vivo* effects of PGJ2 we established a mouse model of neuroinflammation. PGJ2 (16.7ug/2ul/week, for 3 weeks) was bilaterally microinfused into the CA1 hippocampal region of old (53 weeks) and young (12 weeks) mice. Following one week of post-surgery recovery, all mice underwent a 12-day training period on the radial 8-arm maze (RAM) to assess spatial learning and memory. Our data revealed significant spatial learning and long-term memory deficits in old PGJ2-treated mice, compared to the other three groups. Hippocampal Fluoro-Jade C staining identified a significant increase in degenerating neurons in the CA3 region of old PGJ2-treated mice, indicating a progressive spread of damage from the site of injection to the adjacent sub-region. Golgi-immunohistochemical analysis identified a significant deficit in the expression of plasticity-related spine types, stubby and filopodia, within CA1 dendrites of old-PGJ2 treated mice

compared to the young-PGJ2 condition. This deficit occurred concomitantly with a significant increase in colocalization of synaptic markers GluA2/PSD-95 within these spine types, indicating a deficit in maturation of spines via disrupted molecular trafficking and turnover. Our findings indicate that PGJ2, as a product of inflammation, can initiate neurodegeneration as well as facilitate its progression within the hippocampus in an age-dependent manner, thus mimicking processes that are highly relevant to AD pathology. We also assessed the efficacy of elevating endogenous cAMP as a therapeutic intervention in our model. Accordingly, a group of old mice received PGJ2 injections at the same time as PACAP27, a lipophilic neuroprotective peptide that raises intracellular cAMP. PACAP27 treatment ameliorated PGJ2-mediated learning and memory deficits on the RAM. We propose that this pre-clinical mouse model is highly valuable to identify and optimize therapeutics that suppress the neurotoxic effects of inflammation as a strategy to prevent or delay the progression of AD.

Disclosures: **J.A. Avila:** None. **M. Kiprowska:** None. **T. Jean-Louis:** None. **P.A. Serrano:** None. **P. Rockwell:** None. **M.E. Figueiredo-Pereira:** None.

Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 601.07/S5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Canadian Vascular Network

Title: Cerebral vascular pathologies and executive dysfunction in a new animal model of age-related cognitive impairment

Authors: *A. ELHARRAM, N. CZEGLEDY, B. BENNETT, D. ANDREW;
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Abstract: Oxidative stress causes tissue damage in a number of models of vascular cognitive impairment (VCI) and in age-related Alzheimer's disease (AD). We have developed a novel oxidative stress-based mouse model of *age-related* cognitive impairment based on gene deletion of aldehyde dehydrogenase 2 (ALDH2). ALDH2 is important for the detoxification of endogenous aldehydes such as 4-hydroxynonenal (HNE), a lipid peroxidation product formed during oxidative stress that can form protein adducts, altering cell function. $Aldh2^{-/-}$ mice exhibit oxidative stress, and many VCI- and AD-like pathologies including a progressive decline in recognition and spatial memory, increased anxiety- and depressive-like pathologies, aortic

endothelial dysfunction and arterial hypercontractility, HNE adduct formation and age-related increases in amyloid-β (Aβ) in cerebral microvessels, and a loss of blood-brain barrier integrity and cerebral vascular microbleeds. In contrast to AD, VCI has been characterized by early and significant declines in attention and executive function, a term that encompasses a broad range of higher cognitive processes such as: reasoning, planning, cognitive flexibility, sequencing, response inhibition, and abstract concept formation. In order to further evaluate $Aldh2^{-/-}$ mice as a possible model of VCI, attention and executive dysfunction was assessed using the attentional set-shifting task. Cerebrovascular function was also examined using laser Doppler flowmetry, and systolic and diastolic abnormalities of the heart were assessed to determine correlations between cerebrovascular flow and brain pathologies. Further validation of $Aldh2^{-/-}$ mice as an oxidative stress-based animal model of cognitive impairment and age-related VCI will complement current rodent models and will allow greater insight into the pathogenesis and mechanisms of VCI.

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Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 601.08/S6

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Reduced cognitive flexibility and altered metabolic profile in the prefrontal cortex of APPSwDutIowa/$Nos2^{-/-}$ (CVN) mice

Authors: *J. T. PUOLIVALI, A. SHATILLO, K. LEHTIMÄKI, M. KOPANITSA, T. PIIPONNIEMI, T. MIETTINEN, E. VAUHKONEN, P. VARTIAINEN, A. NURMI, P. J. SWEENEY;
Charles River Discovery, Kuopio, Finland

Abstract: The CVN mouse model of Alzheimer’s disease (AD) is characterized by extensive amyloid deposition, expression of hyperphosphorylated tau, and significant neuronal loss. Twelve-month-old CVN mice exhibit impaired spatial memory, as gauged by their inferior performance in the 2-day radial-arm water maze test compared to wild-type (WT) animals. However, their behavior in more translational cognitive tasks has not been thoroughly investigated. We assessed learning and cognitive flexibility of 5-6-month-old CVN mice and their age-matched WT counterparts in a touch screen Visual Discrimination/Reversal (VDR) task, where animals have to learn which one of the two simultaneously displayed stimuli is

correct and subsequently re-learn upon a switch of contingencies. There was no effect of genotype on the number of sessions required to complete instrumental pretraining. Furthermore, CVN and WT animals did not differ in the rate of VD acquisition in terms of the number of days to achieve criterion, number of trials completed, number of errors made, and number of received correction trials ($P < 0.05$ in all comparisons, Mann-Whitney test). However, after the contingency of reward dispensation was switched from the previously correct to previously incorrect stimulus response, CVN mice displayed impaired reversal learning. Analysis of response accuracy across 12 sessions revealed a significant effect of genotype ($F(1,27) = 10.9$, $P = 0.0027$) and session ($F(11,297) = 62.83$, $P < 0.0001$), but no significant interaction between these two factors ($F(11,297) = 0.92$, $P = 0.519$). Furthermore, CVN mice made significantly more errors and received a significantly higher number of correction trials during the 12 reversal sessions. As reversal learning depends on the integrity of prefrontal cortex, we analyzed the metabolic profile in this brain region by 1H magnetic resonance spectroscopy (1H-MRS) using a 11.7 T small animal MRI system. We observed lower levels of the neuronal marker N-acetyl aspartate and glutamate in the brain of CVN mice, whereas myo-inositol concentration was increased. We have previously detected similar changes in the hippocampus of 12-month CVN animals. Furthermore, changes in these metabolites are qualitatively similar to alterations seen in the brain of AD patients. Our results suggest that highly translatable touch screen tests sensitively detect cognitive consequences of AD-like pathology in the brain of relatively young CVN mice. Furthermore, our data indicate that in addition to previously documented hippocampal disturbances, some behavioral impairments in this AD model may be explained by dysfunction of the prefrontal cortex.

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Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NIDCD R01DC014723

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NIH NIDCD R01DC011286

Title: Fus1 KO female mice have olfactory, spatial and association memory impairments and sleep/awake cycle disturbances in adult age: a new model for sAD

Authors: *G. CORONAS-SAMANO[1], K. L. BAKER[1,2], A. V. IVANOVA[3], J. V. VERHAGEN[1,2];
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Abstract: Aging is a complex biological process in humans characterized by a progressive impairment of sensory function, motor skills and cognition over time. Mitochondrial dysfunction has been proposed to play a critical role in the aging process, in which overproduction of reactive oxygen species (ROS) induces oxidative *damage* to cellular macromolecules. Hence, mitochondrial dysfunction has been implicated in chronic and neurodegenerative-disorders such as sporadic Alzheimer's disease (sAD). Fus1/Tusc2 is a mitochondrial tumor suppressor protein regulating numerous mitochondrial activities. Fus1 knockout (KO) in mice results in increased ROS production, mitochondrial dysfunction, chronic inflammation and premature aging. Our group has previously demonstrated that 10 months old (mo.) Fus1 KO female mice showed olfactory dysfunctions in the habituation-dishabituation test and also in the long term memory digging test. Here, we show that Fus1 KO mice is a novel useful animal model for studying accelerated aging. We tested if the Fus1 KO mouse model shows AD related symptoms early in life including olfactory deficits, reference and working memory decline, circadian disruptions, depression and anxiety. We tested female adult Fus1 KO (n=23) and the WT background 129sv (n=14) mice starting from 4 mo. over a 3 month period. Animals were tested for the following behavioral tests: olfactory habituation/dishabituation, short- and long-term memory (all incl. sniffing), hidden cookie, open field, light-dark box, passive avoidance, reduced-stress Morris water maze, circadian sleep/awake cycle, nesting and sucrose preference test. All data were objectively scored using Noldus video analysis. Our data suggests that Fus1 KO mice did not habituate to social odor (urine) over minutes to hours. They did not improve their latency in finding the hidden cookie. In the Morris water maze KO mice did not learn the location of the platform (training session) and did not improve their behavior by the fifth day (testing day). In the light-dark box test, Fus1 KO mice spent more time switching between boxes in comparison with their WT counterparts and showed shorter latency in passive avoidance test. The KO mice slept longer in the dark cycle as compared to WT mice. Fus1 KO mice did not show depression or coherent anxiety symptoms. These findings support the hypothesis that mitochondrial dysfunction in Fus1 KO mice negatively affects cognition and other functions that parallels sAD. Thus, Fus1 KO mice is a useful model for studying aging and aging-associated disorders such as AD characterized by memory decline and increased ROS production.

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Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Bright Focus Foundation to CCK

NIH F31AG050357 to SMN

Title: Novel murine resource to identify genetic modifiers of Alzheimer's disease

Authors: *S. M. NEUNER[1], L. A. WILMOTT[1], T. M. SHAPAKER[1], M. J. HUENTELMAN[2], C. C. KACZOROWSKI[1,2];
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Abstract: Mutations in the amyloid precursor protein or presenilin genes are known to cause familial Alzheimer's disease (FAD). However, there is a wide variation in the age at onset (AAO) of FAD, suggesting additional genes are involved in determining when a patient will develop disease. Uncovering genetic modifiers of the AAO of FAD is of critical importance, as even delaying the onset of symptoms by 5-10 years would significantly reduce the human burden and costs associated with FAD. In order to systematically identify these modifiers, we generated a novel panel of genetically diverse mice harboring five causal FAD mutations in APP and PSEN1 (5XFAD transgene). We have phenotyped this panel, termed the AD-BXDs, across the lifespan and have found that the genetic background of each individual strain has a profound effect on the penetrance of FAD mutations on cognitive traits. Specifically, the AAO of spatial working memory deficits varied widely, ranging from 4 months of age (susceptible strains) to greater than 16 months of age (resilient strains). This translates to approximately 26-60+ years of age in humans, a time frame consistent with the variation in AAO recently observed in human FAD patients (Lee et. al. 2015). In addition, the severity of long-term contextual fear memory deficits at 14 months of age varied, with resilient strains performing on-par with 6-month-old controls while susceptible strains exhibited almost complete memory failure. Through genetic interval mapping, two significant quantitative trait loci containing gene variants that modify AAO and severity of memory deficits were identified. Positional candidates were involved in functions including neuronal nitric oxide signaling and regulation of immune response, providing a novel list of genes that may be effectively targeted to delay the onset of AD. Current work in the lab is aimed at validating a functional role for our top candidates through gain and loss of function studies using viral-mediated gene therapy and genome editing technologies. Since recent work by Lee and colleagues suggest genetic modifiers of AAO in FAD families also

influence AAO in sporadic late-onset AD (LOAD) cases, we hypothesized that genetic modifiers identified here would influence the development of LOAD in human populations. In support, we have identified variants in four of our leading candidates that were significantly associated with LOAD using the TGen2 cohort. Overall, results here are poised to significantly advance our understanding of how individual genetic variation contributes to susceptibility to multiple forms of AD, and demonstrate the utility of the AD-BXD panel as a novel resource for the study of AD genetics.

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Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH F31AG050357 to SMN

Title: Genetic modifiers of non-cognitive symptoms of Alzheimer's disease using a novel transgenic murine panel

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Abstract: Alzheimer's disease, the most common form of dementia in the elderly, often presents with a wide range of non-cognitive comorbidities, including physical frailty, weight loss, and blood glucose regulation (i.e., diabetes). Although a majority of AD research focuses on the neurobiological mechanisms underlying the cognitive deficits seen in AD, these non-cognitive symptoms are often a considerable source of burden for both patients and their caregivers and can drastically affect quality of life. There is evidence that the development of non-cognitive symptoms can be influenced by an individual's genetic makeup, so the identification of precise genes involved is critical both for understanding disease pathophysiology and developing therapeutics to treat symptoms. Our lab recently generated a novel panel of genetically diverse mice harboring five causal mutations in APP and PSEN1 (5XFAD transgene), termed the AD-BXD panel, and has demonstrated that genetic background significantly modulates the penetrance of FAD mutations on cognitive symptoms. We hypothesized that genetic background would also modulate non-cognitive symptoms associated with FAD, so in order to systematically

identify modifier genes, we comprehensively phenotyped AD-BXD mice across the lifespan for a number of non-cognitive traits. Overall, 5XFAD mice exhibited a more severe sensorimotor decline from 6 months of age to 14 months of age and gained less weight then their non-transgenic (Ntg) littermates, reminiscent of the increased physical frailty observed in human AD patients. In addition, 5XFAD mice exhibited a significant decrease in blood glucose levels from 6 months of age to 14 months of age, suggesting an inability to maintain glucose levels. However, the extent of each of these effects was modulated by strain background. Genetic interval mapping identified quantitative trait loci associated with sensorimotor decline, weight curve across the lifespan, and blood glucose levels at 6 months of age, providing a novel list of candidate genes that can potentially be targeted to treat non-cognitive comorbidities associated with FAD. Recent work by Lee and colleagues (2015) has demonstrated that genetic modifiers of the age at onset of FAD also influence the age at onset of sporadic late-onset AD (LOAD), so it reasonable to speculate that genetic modifiers identified here may also influence the development of non-cognitive symptoms associated with LOAD. Overall, results here provide insight into additional symptoms associated with Alzheimer's and are poised to provide a more complete understanding of the genetics of multiple types of AD.

Disclosures: S. Neuner: None. **L. Wilmott:** None. **K. O'Connell:** None. **C.C. Kaczorowski:** None.

Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 601.12/S10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: This research was supported by a donation to the Brown Institute for Brain Sciences

Title: Cognitive deficits in a rodent model of normal pressure hydrocephalus

Authors: *D. L. POETA[1], H. A. BOUNDS[1], I. T. PEREIRA[1], P. M. KLINGE[3], R. D. BURWELL[1,2];
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Abstract: Normal pressure hydrocephalus (NPH) is a cerebrospinal fluid (CSF) disorder resulting from abnormal CSF circulation and its accumulation in the ventricles of the brain. The accumulation of CSF results in enlarged ventricles (ventriculomegaly) and damage to

surrounding brain tissue. NPH patients exhibit cognitive and motor deficits (reviewed in Peterson et al. 2016). Cognitive signs include impairments of memory, attention, and executive function. Motor deficits include alterations in gait. Although these deficits are well documented in NPH patients, there are limited studies addressing the cognitive impairments in animal models of NPH. Previously, the rat kaolin model of adult NPH was shown to cause significant ventriculomegaly and associated brain pathology involving hippocampal structures (Klinge et al, 2003). In the present study, we employed this rat kaolin model to examine the cognitive impairments associated with the disease.
The major goal of the study was to identify a memory task that was sensitive to the kaolin procedure and that was suitable for tracking the emergence of memory deficits during the development of NPH. Thus, we selected a recognition memory task that permits repeated testing, the 3-dimensional spontaneous object recognition (SOR) task. The SOR task has become the preferred experimental paradigm to investigate neurobiological mechanisms of recognition memory in rodents (Ennaceur and Delacour, 1988). In a pilot behavioral study, we compared the performance of 3 control rats with 6 NPH rats on the SOR task at 2, 4, and 6 weeks after the kaolin procedure. Control rats showed normal recognition memory at all time points by exploring the novel object more that the familiar object. This was evident in a positive discrimination ratio (DR) significantly greater than zero. DRs near zero indicate impairment in recognition memory evident in the lack of preference for the novel object. NPH rats showed significantly positive DRs at 2 weeks, but at 4 and 6 weeks DRs were not significantly different from zero indicating deficits in recognition memory. Further confirming the model, NPH rats showed impairments in gait evident in a shorter stride length and a wider base of support compared with control animals. In future experiments, we will employ versions of the SOR task that are sensitive to pathology in the hippocampus, parahippocampal, and prefrontal cortices (Ameen-Ali et al., 2015), regions known to be affected in NPH.

Disclosures: **D.L. Poeta:** None. **H.A. Bounds:** None. **I.T. Pereira:** None. **P.M. Klinge:** None. **R.D. Burwell:** None.

Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

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Program#/Poster#: 601.13/S11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health, USA

the Scientific and Technological Research Council of Turkey (TUBITAK), Project number 111T449

Title: A human carboxypeptidase e/nf-α1 gene mutation in an alzheimer's disease patient leads to dementia and depression in mice

Authors: Y. CHENG[1], N. CAWLEY[1], T. YANIK[2], S. MURTHY[1], C. LIU[1], F. KASIKCI[2], D. ABEBE[1], *Y. LOH[1];
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Abstract: Patients with Alzheimer's disease (AD), a common dementia among the aging population, often also suffer from depression. This comorbidity is poorly understood. Although most forms of AD are not genetically inherited, we have identified a new human mutation in the carboxypeptidase (CPE)/neurotrophic factor-α1 (NF-α1) gene from an AD patient that caused memory deficit and depressive-like behavior in transgenic mice. This mutation consists of three adenosine inserts, introducing 9 amino acids, including 2 glutamines into the mutant protein, herein called CPE-QQ. Expression of CPE-QQ in Neuro2a cells demonstrated that it was not secreted, but accumulated in the endoplasmic reticulum and was subsequently degraded by proteasomes. Overexpression of CPE-QQ in rat hippocampal neurons resulted in cell death, through increased ER stress and decreased expression of pro-survival protein, BCL-2. Transgenic mice overexpressing CPE-QQ did not show difference in the processing enzyme activity of CPE compared with wild type mice. However, the transgenic mice exhibited poor memory, depressive-like behavior, severely decreased neurites in the hippocampal CA3 region and medial prefrontal cortex indicative of neurodegeneration, hyperphosphorylation of tau at Ser^{396}, and diminished neurogenesis in the dentate gyrus at 50 week old. All these pathologies are associated with AD and the latter with depression. Interestingly, the younger CPE-QQ mice (11 week old) did not show deficits in neurite outgrowth and neurogenesis. This study has uncovered a human CPE/NF-α1 gene mutation that could lead to comorbidity of dementia and depression, emphasizing importance of this gene in cognitive function.

Disclosures: Y. Cheng: None. **N. Cawley:** None. **T. Yanik:** None. **S. Murthy:** None. **C. Liu:** None. **F. Kasikci:** None. **D. Abebe:** None. **Y. Loh:** None.

Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 601.14/S12

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Recognition memory in intracerebroventricular streptozotocin-injected rats

Authors: *H. GERGERLIOGLU[1], M. OZ[2], E. DEMIR[3], S. BAGCI-TAYLAN[4], B. YAZGAN[6], H. BARISKANER[4], B. OZTURK[5], G. TEKIN[5];
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Abstract: Improved healthcare comes with a cost of increased prevalence of dementia as the population ages. Alzheimer's disease is the most prevalent cause of dementia in elderly people (2). To study the neuropathology resembling Alzheimer's disease in laboratory animals, intracerebroventricular (ICV) streptozotocin (STZ) injection is a commonly used method which engenders several pivotal molecular traits of this malady such as oxidative stress, neuroinflammation, amyloid plaque formation, and tauopathy (3). In the present study, we evaluated recognition memory in ICV STZ-injected Wistar albino rats by using a novel object recognition task which enables estimation of global (concurrently cortical and hippocampal) short-term and long-term memory as well as the memory retention. To this aim, two groups (n=8 in each) of animals received either artificial cerebrospinal fluid or STZ (3 mg/kg). Two weeks after this intervention, the recognition test, comprising a habituation phase, in which animals are left to move freely for 5 minutes, and 3 diverse 5-minute-long test phases as familiarization, short-term memory, and long-term memory, was performed. Data were analyzed by two-tailed Student's t-test and $p<0.05$ was considered significant. Two identical objects were used in the familiarization phase, and no place preference was observed ($p>0.05$). One of the objects was changed with the novel object after one hour (short-term phase). ICV STZ-injected rats displayed significantly less exploratory behavior around the novel object than the controls ($t=6.99$, $df=14$, $p<0.01$) which indicates impaired short-term memory. Twenty-four hour after the familiarization, in the long-term memory phase, the familiar object was placed onto the other location and a different novel object was put on the previously familiar location to eliminate spatial cues. Interestingly, there was no statistical significance between the animals in this phase ($p>0.05$). According to these results, we assume that ICV STZ administration did not disrupt memory acquisition (working memory in other words) that is interpreted by preserved long-term memory, but it devastated short-term memory retention. However, this finding seems to be related to a difficulty of memory recall rather than of short-term memory storage.
References:
1-Antunes M & Biala G. (2011). The novel object recognition memory: neurobiology, test procedure, and its modifications. Cogn Process, 13(2), 93-110. DOI: 10.1007/s10339-011-0430-z
2-Ferencz B & Gerritsen L. (2015). Genetics and Underlying Pathology of Dementia. Neuropsychol Rev, 25(1), 113-124. DOI: 10.1007/s11065-014-9276-3

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Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

Location: Halls B-H

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Program#/Poster#: 601.15/S13

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 1R01GM104194

William K Hamilton Endowment Fund, UCSF

Title: Surgery causes exaggerated postoperative cognitive decline in high fat diet induced obese mice

Authors: *X. FENG[1], M. V. CONTRERAS[2], S. KOLIWAD[2], M. MAZE[3];
[1]Anesthesia and Perioperative Care, [2]Med., [3]Anesthesia and Perioperative care, Univ. of California, San Francisco, San Francisco, CA

Abstract: Introduction: We have demonstrated that neuroinflammation following surgery produced cognitive decline in previous studies (1-4). Furthermore, we have identified deficiencies in the resolution of inflammation induced by aseptic trauma in the rat model of metabolic syndrome (5,6). In this study we would like to investigate whether surgery could cause exaggerated postoperative cognitive decline in high fat diet induced obese (DIO) mice Methods: Young mice (5-6 weeks old) were fed with high fat diet or control diet for 8 weeks before surgery (tibial fracture) till sacrifice. For the behavioral study, trace fear conditioning (TFC) training was performed 30 minutes before surgery, and TFC testing was performed on the 3rd day postoperatively. Mice were sacrificed 3 days postoperatively, plasma and hippocampus were harvested for detecting LXA4 and LTB4, and neuroinflammation, respectively. Results: Postoperative cognitive decline was exaggerated in DIO mice. High fat diet induced much high neuroinflammatory response to surgery and decreased systemic LXA4 level. Discussion: The neuroinflammatory and cognitive responses to peripheral surgery are exaggerated in DIO mice. DIO mice may be a useful preclinical model in which to investigate therapeutic strategies to prevent and/or ameliorate exaggerated postoperative cognitive decline and other postoperative complications in which inflammation fails to resolve.

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Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Midkine modulates striatal gliosis and cognitive impairment induced by amphetamine: Evidence for a stimulus-dependent regulation of neuroinflammation by midkine

Authors: *G. HERRADON, R. FERNÁNDEZ-CALLE, C. PÉREZ-GARCÍA, E. GRAMAGE, M. VICENTE-RODRÍGUEZ;
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Abstract: Midkine (MK) is a cytokine with important functions in inflammatory processes in peripheral organs in different pathological conditions. Despite amphetamine-induced striatal astrogliosis, a hallmark of this drug's neurotoxic effects, is increased in MK knockout (MK-/-) mice, the possible role of MK in neuroinflammation is not yet fully understood. To fill this gap, we have studied astrogliosis and microglia activation induced by amphetamine in MK-/- and wild type (WT) mice. Animals received 4 injections (i.p.) of amphetamine (10 mg/kg) or saline (control, 10 ml/kg), allowing between injections a 2 hour interval. Four days after last amphetamine injection, astroglia and microglia were examined in immunohistochemistry studies

using antibodies against GFAP and Iba-1, respectively. We found that amphetamine-induced microglial response and astrocytosis are significantly enhanced in the striatum of MK-/- mice. This counteractive effect of MK against amphetamine striatal gliosis seems to be region-specific since it is not observed in the hippocampus. To test the possibility that MK regulates microgliosis and astrocytosis independently of the inflammatory stimulus, we assessed the effects of lipopolysaccharide (LPS, 0.5 mg/kg, i.p.) in MK-/- and WT mice. Surprisingly, LPS-induced striatal astrogliosis was blocked by genetic inactivation of MK, suggesting a differential regulation of astrocytosis by MK depending on the inflammatory stimulus. Since early onset of amphetamine-type drugs abuse is associated with a wide range of adverse outcomes in adulthood including cognitive deficits, we also tested long term effects of periadolescent amphetamine treatment (3 mg/kg i.p., daily during 10 days) in a memory task in MK-/- and WT mice. Within two weeks after cessation of treatment, significant deficits in the Y-maze test were only observed in amphetamine-pretreated MK-/- mice. However, 26 days after last administration we did not find significant differences between genotypes. The data presented here demonstrate for the first time that MK is an important modulator of amphetamine-induced striatal neuroinflammation. The data also suggest that the modulatory effects of MK in neuroinflammation depend on the inflammatory stimulus and the brain area considered. In addition, our data demonstrate that periadolescent amphetamine treatment in mice results in transient disruption of learning and memory processes in absence of endogenous MK.

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Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

Location: Halls B-H

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Program#/Poster#: 601.17/T1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: KMU-TP104D05

KMU-Q104013

Title: Human very low density-lipoprotein induces neuronal inflammation and cognitive dysfunction in mice

Authors: *S.-L. CHEN[1], H.-C. LEE[1], M.-C. CHOU[1], L.-Y. KE[1], C.-L. LAI[1], C.-H. CHEN[2], C.-K. LIU[1];
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Abstract: Present epidemiologic findings indicate that dyslipidemia is associated with accelerated onset and progression of neuronal degeneration and cognitive impairment. Abnormal lipids, in particular electronegative very low density lipoprotein (VLDL), which circulates abundantly in the plasma of patients with the metabolic syndrome (MetS), is toxic to microvascular endothelial cells. We hypothesize that plasma VLDL from MetS patients (MetS-VLDL) plays a role in neurodegeneration by eliciting inflammation and damage of the neuronal tissue. To test the hypothesis, MetS-VLDL and VLDL isolated from normal control subjects (nVLDL) were used in both *in vitro* and in *vivo* settings.
In primary microglial cultures, addition of MetS-VLDL but not nVLDL (both 5 μg/mL) induced significant TNF-α secretion and microglial activation within 24 hours. In wild-type B6 mice, daily tail-vein injections of nVLDL or MetS-VLDL (15 μg/g/3 times/week) induced significant cognitive dysfunction in 6 weeks, as evaluated by Y-Maze task (n=12, P=0.027). In medial prefrontal cortex brain sections, mild to moderate microglial activation was found in both nVLDL (118%, P=0.053) and MetVLDL (135%, P<0.0001) groups, compared with saline treatment. Moreover, the number of activated macrophages adjacent to brain microvessels was increased mildly by nVLDL (120%, P<0.05) and MetS-VLDL (171%, P<0.0001). These data suggest that long-term exposure to excessive human VLDL can induce perivascular microglial inflammation associated with concomitant cognitive dysfunction in mice. MetS-VLDL, which is rich in electronegative VLDL subfractions, exacerbates the pathological changes of neuronal inflammation. Our findings provide new insights into the link between lipid abnormality and neurodegeneration.

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Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 601.18/T2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: WRIISC

Lumind Foundation

Title: Synergistic effects of BDNF-TrkB and Norepinephrine signaling in treating cognitive dysfunction in a mouse model of Down syndrome

Authors: *R. PONNUSAMY[1], B. MEDINA[2], K. SUMANTH[2], S. MOGHADAM[2], H. BAKKER[2], M. BAKTIR[2], F. S. MOJABI[2], A. FAHIMI[2], M. W. MCNERNEY[1], A. SALEHI[2,3]; [1]Psychiatry, VA Palo Alto, Palo Alto, CA; [2]Psychiatry, Palo Alto Veterans Inst. For Res., Palo Alto, CA; [3]Psychiatry, Stanford Sch. of Med., Palo Alto, CA

Abstract: Down syndrome (DS) or trisomy of human chromosome 21, affects around 400,000 individuals in the US. In addition to varying degrees of learning disability, all adults with DS will develop Alzheimer's disease (AD)-related pathology by the age of 40 years. This makes DS a good model to study molecular mechanisms of cognitive dysfunctions in AD. Previously, we provided evidence that increasing brain norepinephrine (NE) levels and/or improving β2 adrenergic signaling can restore cognitive function in the Ts65Dn mouse model of DS. Moreover, a number of recent studies suggest that there is an interaction between Beta adrenergic receptors and BDNF-TrkB signaling. We hypothesized that increasing Beta adrenergic signaling along with BDNF-signaling can have synergic effects and thus more effectively mitigate cognitive dysfunction. Adult Ts65Dn mice and their controls were given 8 weeks of physical exercise (PE) on a treadmill with or without a long acting β2 adrenergic agonist. These mice then were subjected to various behavioral testing procedures including open field, novel object recognition, and contextual fear conditioning. We found that combining PE and improving NE signaling in Ts65Dn mice had far more superior effects on cognitive function than either treatment alone. Our results provide an unprecedented opportunity to develop a new therapeutic strategy for restoring cognitive functions in both DS and AD.

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Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 601.19/T3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: VA Merit Review

American Federation for Aging Research

Title: Metformin improves memory in diabetic mice and the SAMP8 mouse model of Alzheimer's disease

Authors: *S. A. FARR[1], M. L. NIEHOFF[2], M. W. BERGIN[2], E. C. ROESLER[2], R. KOEHLER[2], G. N. SHAH[3], J. E. MORLEY[4];
[1]St Louis Univ/VA Med. Ctr., Saint Louis, MO; [2]Intrnl. Medicine/Geriatrics, [3]Intrnl. Medicine/Endocrinoloty, [4]Intrnl. Medicine/Geriatrics/Endocrinology, St. Louis University-SOM, St. Louis, MO

Abstract: BACKGROUND
There is a growing body of evidence showing an increased risk of Alzheimer's disease (AD) in people with diabetes. Diabetes and AD have common features including abnormal protein processing, abnormalities in insulin signaling, dysregulated glucose mechanism, oxidative stress, the formation of advanced glycation end products, and activation of inflammatory pathways. Metformin, a mainstay of diabetic treatment, may play a neuroprotective role through increased neuronal viability, protection against oxidative imbalance, and attenuation of AD-like pathology. The purpose of the current study was to determine if metformin had an effect on learning and memory in diabetic mice and in the SAMP8 mouse model of AD.
METHODS
In study 1, we examined the effect of metformin on cognition in diabetic mice. Ten week old CD-1 mice were given streptozotocin (150/mg/kg/i.v) to induce diabetes and then treated with metformin for 8 weeks. Behavioral testing started after 4 weeks of treatment. Mice were tested in T-maze foot shock avoidance, novel object recognition and Barnes maze. In study 2, we examined the effect of metformin on learning and memory in the SAMP8 mouse model of AD. Starting at 11 months of age SAMP8 mice were administered metformin daily for 8 weeks. After 4 weeks of treatment the mice were tested in T-maze foot shock avoidance, object recognition and Barnes maze. At the end of treatment brain tissue was collected for mitochondrial BAX analysis.
RESULTS
In study 1, metformin improved T-maze acquisition at both doses and T-maze retention at 200 mg/kg compared to vehicle treated diabetic mice. The mean time spent exploring the novel object was also significant for both doses of metformin in the novel object recognition task. In the acquisition phase of the Barnes maze, treatment with both doses of metformin showed significant improvement in time to target compared to vehicle treated diabetic mice. In study 2, metformin, 20 mg/kg and 200 mg/kg, improved both acquisition and retention in T-maze foot shock avoidance, retention in object recognition, and acquisition in the Barnes maze (20mg/kg). Metformin improved mitochondrial BAX at 20 mg/kg.
CONCLUSIONS
The current studies show that treatment with metformin in STZ-induced diabetic CD-1 mice and in the SAMP8 mouse model of AD was associated with improved learning and memory compared to their respective vehicle treated control mice, suggesting that metformin may play a neuroprotective role in both diabetes and AD. Metformin also decreased mitochondrial BAX in the SAMP8 mice suggesting a positive effect on the mitochondria. The current results suggest that metformin may have therapeutic potential for AD.

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Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 601.20/T4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 2R15NS060117-02

Title: Probing the effects of kale, arugula, and dandelion greens on the hippocampus and memory in obese pre-diabetic C57BL/6 mice.

Authors: ***B. TENG**[1], D. FOSTER[1], L. BANNER[2];
[2]Biol., [1]California State Univ. Northridge, Northridge, CA

Abstract: The United States is facing a diabetes epidemic. As the 7th leading cause of death and afflicting nearly 10% of the population, diabetes is well studied and its complications are conspicuous, including a battery of neurodegenerative diseases such as Alzheimer's and other cognitive degradation. Diet-induced obesity is an increasingly common occurrence that predisposes individuals to type-2 diabetes and contributes to complications including those of the nervous system and the immune system. Increased body mass is associated with an elevated risk for neurodegeneration and dementia and changes in hippocampal plasticity and spatial learning among others have been documented. While the exact mechanism has yet to be fully understood, neuro-inflammation is thought to be an important factor in impaired cognitive function. Cytokines involved in the inflammatory response are elevated in brains of animals fed a high fat diet (HFD) and a variety of anti-inflammatory/anti-oxidant treatments can reduce this expression and alleviate cognitive changes. What is not well understood is how the progressive nature of cognitive degradation, as measured by memory loss, manifests itself early in the pre-diabetic stage. Cognitive degradation may be ascribed to neuro-inflammation and differences in diet may either aggrandize or temper its severity. Specifically, we are interested in seeing if adding kale, arugula, or dandelion to an existing obesity inducing diet may taper neuro-inflammation and thus stymie cognitive decline.
To address this issue, C57BL/6 mice were fed either a control (10% fat) or high-fat diet (HFD)(60% fat) for 16 weeks until the high-fat group reached a pre-diabetic stage. After 16 weeks, mice on a HFD weighed significantly more than the control mice, displayed elevated blood glucose levels, and showed deficits in spatial learning. During weeks 17 to 25, the diets of

all the mice were supplemented daily with 1.0 gram of fresh kale, arugula, or dandelion. Consumption of the greens had no effect on the weights of either group. During the green diet period the mice were subjected to multiple repetitions of memory tests, such as the Morris Water Maze, Barnes Maze, and Social Recognition Test, to test for changes in their memory abilities. Our preliminary data from the Morris Water Maze demonstrates that animals fed a high fat diet supplemented with arugula or dandelion performed much better than those on a high fat diet alone or HFD plus kale. At 25 weeks the hippocampi were removed and are being analyzed for a variety of inflammatory and neuronal markers.

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Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

Location: Halls B-H

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Program#/Poster#: 601.21/T5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R21AG048446

UTHSC Neuroscience Institute

Title: Reduced hippocampal functional connectivity with prefrontal cortex during a spatial task in an AD mouse model

Authors: ***S. DING**[1], S. M. NEUNER[1], L. A. WILMOTT[1], T. M. SHAPAKER[1], K. M. S. O'CONNELL[1,2], C. C. KACZOROWSKI[1,2];
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Abstract: Both hippocampus (Hip) and medial prefrontal cortex (mPFC) play a critical role in learning and memory. The functional connectivity between the Hip and PFC is thought to be critical for working memory performance and formation of long-term memories, as increased synchronization of neural oscillations of Hip and PFC local field potential (LFP) signals strongly correlate with task performance. Human Alzheimer disease (AD) patients exhibit reductions in the functional connectivity of Hip with other memory relevant brain regions, however, it is unclear whether these changes are a cause or consequence of disease. The 5XFAD mouse model of AD recapitulates deficits in learning and memory observed in AD patients, including deficits in spatial working memory, although changes in functional connectivity in this model have not been examined. We hypothesized that, like human AD patients, Hip-mPFC coherence is

disrupted in the 5XFAD model. To test this hypothesis, custom designed shank electrodes were implanted into Hip and mPFC (both prelimbic cortex (PL) and infralimbic cortex (IL)) of 8-10 month old sex-balanced 5XFAD and wild-type (WT) mice. Following five days of post-surgical recovery, LFP signals of Hip and mPFC were recorded while they were awake and resting quietly in their home cage (resting state) or on the Hip-dependent T-maze task. Hip-mPFC coherence of AD and WT mice was compared in different LFP frequency bands: delta (1-3Hz), low theta (3.01-7Hz), high theta (7.01-12Hz), beta 1 (12.01-20Hz), beta 2 (20.01-30 Hz), and gamma (40-60Hz). Analysis of our results indicated that during the resting state, there was no significant difference in the Hip-mPFC coherence across all frequency bands measured in WT and AD mice. However, the task-specific increase in the coherence of Hip-mPFC in delta and theta frequency bands in WT mice was significantly reduced in age-matched AD mice. These results demonstrate that the 5FXAD model adequately recapitulates deficits in Hip-mPFC coherence observed in human AD patients. Ongoing experiments in the laboratory aim to establish whether these changes occur prior to or after the onset overt memory deficits, and to determine mechanisms mediating changes in Hip-mPFC functional connectivity.

Disclosures: S. Ding: None. **S.M. Neuner:** None. **L.A. Wilmott:** None. **T.M. Shapaker:** None. **K.M.S. O'Connell:** None. **C.C. Kaczorowski:** None.

Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 601.22/T6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: IHDCYH Grants #126790

IHDCYH Grants #136908

NSERC RGPIN-2014-06089

Title: Non-invasive early detection of neuroprotection by high-field MRI in periventricular leukomalacia: a preclinical study

Authors: *W. C. PIERRE[1,2], L. AKAKPO[3], I. LONDONO[1], F. LESAGE[3], P. POULIOT[3], G. A. LODYGENSKY[1,2];
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Montréal, Montreal, QC, Canada; [3]Electrical Engin., École Polytechnique de Montréal, Montreal, QC, Canada

Abstract: Introduction: Preterm inflammation-induced white matter injury (WMI) is associated with well-known neurocognitive impairments. Early monitoring of neuroprotection with a non-invasive tool such as magnetic resonance imaging (MRI) could greatly improve the development of therapeutic approaches. Among neuroprotective agents, Anakinra, an antagonist of interleukin-1 receptor (IL-1Ra), has previously showed beneficial effects in an animal model of WMI. Objective: Using diffusion tensor imaging (DTI) as a non-invasive monitoring tool, we evaluated the early neuroprotective effect of IL-1Ra in neonatal rats subjected to WMI. Methods: P3 Rat pups received LPS (1mg/kg) or sterile saline injections in the corpus callosum. A subset was treated with three i.p. injections of IL-1Ra (2 mg/kg). 24h post-injection, 13 animals (7 sham, 3 LPS, 3 LPS+IL-1Ra) were imaged on a 7 tesla scanner. DTI was acquired using spin echo standard sequence with dual scheme with b-values of 0 and 700 s/mm^2 with a voxel resolution of 230 x 120 x 600 µm. ROIs were placed on the ipsilateral cingulum. Kruskal-Wallis test was used for statistical comparisons. Results are normalized with the sham group. Apoptosis and inflammatory status were assessed in the three groups. Results: In accordance with previous results (Lodygensky et al. Pediatric Res 2014), LPS injection causes a decrease in diffusivity in the acute phase. This decrease is significantly limited for animals that underwent IL-1Ra injections. Fractional anisotropy did not come out as a reliable biomarker for assessing acute WMI. Primary blind qualitative evaluation of apoptosis revealed a sharp decrease in fractin expression in treated animals. Furthermore, IL-1Ra treatment decreased RNA expression of pro-inflammatory genes iNOS, IL-1 and IL-6. Conclusion: DTI allowed early detection (in the 24h post-injury) of neuroprotective effect of IL-1Ra which correlated with a reduction of pro-inflammatory gene expression. More animals are currently being scanned with thorough quantitative immunohistochemistry analysis and evaluation of pro- and anti-inflammatory gene expression. Our results suggest MRI is a non-invasive tool allowing early monitoring of therapeutic response in neonates.

Table 1:ROIs analysis for the different diffusion scalar maps extracted from the diffusion tensor.

DTI Scalar Map	LPS (n=3)	LPS+IL-1Ra (n=3)	p-value
Mean Diffusivity	-42.38 %	-24.14 %	**0.0258**
Axial Diffusivity	-38.04 %	-20.34 %	**0.0129**
Radial Diffusivity	-46.32 %	-27.61 %	**0.0336**
Fractional Anisotropy	48.73 %	45.99 %	0.2893

Disclosures: W.C. Pierre: None. **L. Akakpo:** None. **I. Londono:** None. **F. Lesage:** None. **P. Pouliot:** None. **G.A. Lodygensky:** None.

Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 601.23/T7

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Liraglutide ameliorates intracerebral insulin resistance in "Brain Diabetes" rats

Authors: *A. S. SHINGO[1], T. KANEBAYASHI[2], S. KITO[3], T. MURASE[1];
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Abstract: Intracerebrally streptozotocin injected rats (STZ-3V rats), what we call "Brain Diabetes" rats, show impaired spatial cognition and are considered a model of Alzheimer's disease. On the other hand, it has been established that insulin receptors are widely distributed throughout the brain including the hippocampus. Besides, insulin biosynthesis within the brain, especially in the hippocampus has been confirmed. In this study, we examined phosphorylation state of IRS-1(insulin receptor substrate-1) in the STZ-3V rat hippocampus and effects of liraglutide (a GLP-1 receptor agonist) on the IRS-1 phosphorylation. As methods, phosphorylation state of IRS-1 in the hippocampus of STZ-3V rats was examined by immunohistochemistry before and after 0.037ug/kg liraglutide administration. As the results, in the hippocampus of STZ-3V rats, a decrease of tyrosine phosphorylation accompanied by an increase of serine phosphorylation was observed. Administration of liraglutide induced improvement of tyrosine/serine phosphorylation ratio. It is concluded that in the "Brain Diabetes" rats there was an elevation of insulin resistance that was inhibited by liraglutide administration. The results suggested possibility of liraglutide as a therapeutic drug against Alzheimer's disease.

Disclosures: **A.S. Shingo:** None. **T. Kanebayashi:** None. **S. Kito:** None. **T. Murase:** None.

Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 601.24/T8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSFC Grant 81371223

NSFC Grant 81571061

Title: Suppress sodium channel activity as a new potential therapeutic target for Alzheimer's disease

Authors: *S. LI[1], X. WANG[1], C. Y. JIANG[1], X. G. ZHANG[1], Q. H. MA[2], J. ZHAO[1];
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Abstract: Aberrant increases in neuronal network excitability may contribute to the cognitive deficits in Alzheimer’s disease (AD). However, the mechanisms underlying hyperactivity are not fully understood. Using patch clamp techniques, we found elevated neuronal excitability due to modulation of the voltage-gated sodium channel by amyloid beta (Aβ1-42). Immunohistochemistry and western blot results showed that the intrinsic neuronal hyperexcitability of human amyloid precursor protein (APP) / presenilin 1 (PS1) transgenic mice might be due to an increased expression of voltage-dependent sodium channels subtype. Blocking the overexcitation of the neural network with lamotrigine (LTG), which is a sodium channel blocker applied in a broad-spectrum treatment of epilepsy, suppressed abnormal spike activity, prevented the loss of spines, synaptophysin immunoreactivity, and neurons, and thus attenuated the deficits in synaptic plasticity and learning and memory in APP/PS1 transgenic mice. Scorpion venom heat-resistant peptide (SVHRP) extracted from nature product Buthus martensii Karsch (BmK) venom could rescue the learning and memory deficits in AD animal model. The mechanism might be related with suppressing sodium channel currents and inhibiting the neuronal excitability. Moreover, the levels of brain-derived neurotrophic growth factor (BDNF) were enhanced in the brains of APP /PS1 mice by the chronic both SVHRP and LTG treatment. Therefore, these observations demonstrate that LTG or SVHRP attenuates AD pathology through multiple mechanisms, including modulation of abnormal network activity, reduction of the generation of amyloid beta and upregulation of BDNF. This study has brought sodium channel blocker as a new strategy, especially the potential application of antiepileptic drugs (AEDs), in AD therapy.

Disclosures: **S. Li:** None. **X. Wang:** None. **C.Y. Jiang:** None. **X.G. Zhang:** None. **Q.H. Ma:** None. **J. Zhao:** None.

Poster

601. Assessment and Modulation of Cognitive Phenotypes in Murine Models of Neurodegenerative Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 601.25/T9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 5R01HL122472

Title: The impact of midazolam on the expression of the circadian rhythm protein Per2

Authors: *J. J. GILE, D. SEHRT, B. SCOTT, T. ECKLE;
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Abstract: Critical care units are a major cause of a disrupted circadian rhythm in patients. Interestingly, circadian disruption is frequently associated with the occurrence of delirium having a high impact on outcome and mortality in the critically ill. It has been shown that prolonged exposure to benzodiazepines (such as midazolam) and, to a lesser extent opiates, contributes to the development of delirium. Here we tested the effect of midazolam and other sedatives on the expression of the circadian rhythm protein Period 2 in different organs from mice. All animal experiments were performed in accordance with the APS/NIH guidelines for the use of laboratory animals. Expressional levels of Per2 were assessed by real-time RT-PCR in different organs from mice using midazolam, propofol, fentanyl, ketamine or isoflurane. Midazolam caused a significant downregulation of Period 2 in all organs investigated. The other sedatives had no effect or little on Per2 expression with the exception of ketamine, which increased Per2 in the lung and decreased Per2 in the liver. Midazolam consistently downregulates Per2 in different mouse organs. As midazolam has been found to play a critical role in the development of delirium we suggest that midazolam mediated downregulation of Per2 represents a potential mechanism in the development of delirium. Further studies using a mouse model for delirium will be necessary to further elucidate the underlying mechanism.

Disclosures: J.J. Gile: None. **D. Sehrt:** None. **B. Scott:** None. **T. Eckle:** None.

Poster

602. Rodent Models and Therapeutics in Parkinson's Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 602.01/T10

Topic: C.03. Parkinson's Disease

Support: Universidad Nacional Autónoma de México Porject 080 2012

SIP Project 20160469

Title: Gross Motor Skills, PET imaging study and trajectory analysis of hemiparkinsonian rats after dopamine supply restitution through a TiO_2DA implant placed in striatum.

Authors: *D. A. VÁZQUEZ MATÍAS[1], G. VALVERDE AGUILAR[3], R. MAYEN DÍAZ[2], H. GONZÁLEZ SÁNCHEZ[2], S. HERNANDEZ CASTRO[2], A. MONZÓN MIRELES[2], P. VERGARA ARAGÓN[2];
[1]UNAM, Coyoacán, Mexico; [2]UNAM, Coyoacan Mexico City, Mexico; [3]CICATA Legaria Inst. Politecnico Nacional, Mexico City, Mexico

Abstract: The aim of this study was to assess the effect produced by the placement of a dopamine releasing reservoir in the striatum of hemiparkinsonian rats on the gross motor alterations, imaging evaluation, and trajectory behavior. Methods: 50 male Wistar rats divided in four groups: A) 8 Sham rats, B) 12 hemiparkinsonian rats induced by administration of the 6-OHDA neurotoxin (8µg/4µl) via stereoscopic surgery in left substantia nigra (Lx), C) 20 hemiparkinsonian rats induced alike Lx rats, and later placement of a dopamine reservoir made of titanium dioxide (Lx+TiO2DA) and D) 10 rats only with placement of the dopamine reservoir (TiO2DA). 180 days after hemiparkinsonism induction in the groups Lx and Lx+TiO2DA, all the groups underwent to apomorphine induced rotational test to evaluate the damage in the dopaminergic system, gross motor performance and path were analyzed in the open field test. Positron Emission scans were performed on left and right striatum measuring the uptake of the tracer 11[C]-DTBZ to obtain a measurement of the nigrostriatal pathway. Once finalized the evaluations the data was analyzed by one-way ANOVA and multiple comparisons Tukey's test; p values < 0.001 were considered significant. Results: Apomorphine rotational behavior showed an increased number of spins in Lx compared to Sham, Lx+TiO2DA and TiO2DA Groups: 295.2 ± 10.1 vs 4.4 ± 0.8, 30.1±4.2 and 11.0±2.9. Lx+TiO2DA and TiO2DA do not present differences compared to the Sham Group. Open field test revealed a decrease in gross motor activity assessment in Lx animals vs Sham, Lx+ TiO2DA and TiO2DA groups: 16.1±2.8 vs 106.5±4.6, 97.5±7.6 and 122.2±5.8. Lx+TiO2DA and TiO2DA do not present differences compared to the Sham Group. At the day 180, Sham animals follow a path close to the open field walls, Lx animals either move to the center of the field or remain immobile in one place. Lx+TiO2DA use to move surrounding the field and visit the center occasionally. TiO2DA animals move fast

around the field and usually visit the center. PET scans disclosed on the left striatum an increase of uptake in the injured animals compared to implanted and Sham animals. The right striatum displayed the same pattern. Conclusion: This study has revealed an effective dopamine supply restitution by the implantation of a titanium dioxide dopamine reservoir, which is evident by the improvement of Lx+TiO2DA animal's performance in the rotational behavior and open field test; as well as, an enhancement demonstrated on 11[C]DTBZ uptake in Lx+TiO2DA animals, which suggest a recovery in the dopaminergic striatal innervation.

Disclosures: D.A. Vázquez Matías: None. **G. Valverde Aguilar:** None. **R. Mayen Díaz:** None. **H. González Sánchez:** None. **S. Hernandez Castro:** None. **A. Monzón Mireles:** None. **P. Vergara Aragón:** None.

Poster

602. Rodent Models and Therapeutics in Parkinson's Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 602.02/T11

Topic: C.03. Parkinson's Disease

Support: Michael J Fox Foundation

Bagadilico

Multipark

Vetenskapsrädet

Title: Regulated GDNF delivery shows transient neuroprotective effect

Authors: *L. QUINTINO[1,2], L. BREGER[2], M. LUNDBLAD[2], C. ISAKSSON[2], C. LUNDBERG[2];
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Abstract: Glial cell-line derived neurotrophic factor (GDNF) is a therapeutic factor for the treatment of Parkinson's Disease (PD). However, overexpression of GDNF in the brain causes side-effects. Our group has used destabilizing domain technology to induce GDNF (GDNF-DD) expression in vivo. Induction of gene expression would enable GDNF to protect neurons while turning off the expression of GDNF would prevent side-effects. The aim of this study was to determine how long the neuroprotective effect of GDNF-DD could be maintained after GDNF-DD expression was turned off. To achieve this, lentiviral vectors expressing GDNF, GDNF-DD or regulated yellow fluorescence protein (YFP-DD) were delivered to the striatum of rats. Three

days after vector delivery, GDNF-DD and YFP-DD expression was turned on for 9 weeks. Three weeks after vector delivery the animals were lesioned with 6-OHDA. Groups of animals were analysed for behaviour, amperometry and their brains were processed for histology at several timepoints. Six weeks after lesion, while GDNF-DD expression was induced the GDNF group had 75% remaining neurons in the substantia nigra (SN). The GDNF-DD group had 50% remaining neurons whereas the YFP-DD group had only 18% remaining neurons in SN. While at later weeks, when expression was turned off, the GDNF group maintained the level of neuroprotection, while the GDNF-DD group lost the neuroprotective effect to levels comparable to the YFP-DD group This study indicates that while constant overexpression of GDNF has deleterious effects, there needs to be a fine regulation of GDNF to maintain the therapeutic effect.

Disclosures: **L. Quintino:** None. **L. Breger:** None. **M. Lundblad:** None. **C. Isaksson:** None. **C. Lundberg:** None.

Poster

602. Rodent Models and Therapeutics in Parkinson's Disease

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Program#/Poster#: 602.03/T12

Topic: C.03. Parkinson's Disease

Support: INNPACTO Subprogram IPT-2012-1208-300000

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Instituto de Salud Carlos III PI13/01390

European Regional Development Fund "A way to build Europe"

Title: Antisense oligonucleotide reduction of human alpha-synuclein accumulation in dopamine and serotonin neurons prevents early dysfunctions in a mouse model of Parkinson's disease

Authors: D. ALARCON-ARIS[1], E. RUIZ-BRONCHAL[1], A. MONTEFELTRO[2], F. ARTIGAS[1], *A. BORTOLOZZI[3];
[1]IIBB - CSIC - IDIBAPS, Barcelona, Spain; [2]nLife Therapeut., Granada, Spain; [3]IDIBAPS, Barcelona, Spain

Abstract: Multiple convergent lines of evidence implicate α-synuclein (encoded by *SCNA*) in the pathogenic of Parkinson's disease (PD). α-Synuclein is a protein that accumulates in the brain of patients with sporadic PD. Likewise, whole locus multiplications and point mutations in

the α-synuclein gene cause a familial form of PD. Previously, we developed a mouse model that overexpress wild-type human-α-synuclein in the AAV5 vector (donated by MJF foundation) in dopamine (DA) neurons of the substantia nigra compacta (SNc) and ventral tegmental area (VTA) as well as in serotonin (5-HT) neurons of the raphe nuclei (RN). These mice showed increased human-α-synuclein mRNA levels in the ipsilateral SNc (278% of sham mice) and RN (290% of sham mice), but they did not display any loss of tyrosine hydroxylase-positive DA neurons or tryptophan hydroxylase-positive 5-HT neurons at 16 weeks post-injection. Moreover, reduced DA and 5-HT release paralleled with development of α-synuclein-positive axonal swelling in striatum, hippocampus and cerebral cortex were found in the AAV5 model. In addition, AAV5 mice exhibited motor deficits at 8 weeks post-infection into SN and depressive-like behaviors (tail suspension and forced swim tests) at 4 weeks post-infection into RN. Here, we evaluated whether reducing human α-synuclein expression in the mesencephalic nuclei prevented early dysfunctions in the AAV5 model. We used a conjugated antisense oligonucleotide targeting intracellular human α-synuclein (ASO1337) selectively in DA and 5-HT neurons. Mice overexpressing human α-synuclein in the SNc/VTA or RN were treated intracerebroventricularly with ASO1337 (30 or 100µg/day) during 28 days using osmotic minipumps implanted subcutaneously. Control groups received vehicle or nonsense ASO sequence (ASO1227) in the same conditions. ASO1337 reduced dose-dependent the human α-synuclein mRNA levels (vehicle: 0.76±0.04; ASO1337 30µg/day: 0.46±0.05; ASO1337 100µ/day: 0.35±0.03, arbitrary units), whereas endogenous α-synuclein and γ-synuclein expression remained unaltered. Immunohistochemistry analysis confirmed these results showing the reduction of human α-synuclein protein density. Furthermore, recovery of striatal DA release could be achieved by reduction of human-α-synuclein expression in SNc/VTA. Recent data showed that intracerebroventricular ASO1337 infusion (100µg/day, 28 days) also decreased human α-synuclein mRNA expression in the RN of AAV5 mice compared to control groups. Our study indicated that the ASO-induced reduction of intracellular α-synuclein accumulation selectively in DA and 5-HT neurons using ASO molecules represents an optimal PD therapy.

Disclosures: **D. Alarcon-Aris:** None. **E. Ruiz-Bronchal:** None. **A. Montefeltro:** A. Employment/Salary (full or part-time): nLife Therapeutics. **F. Artigas:** None. **A. Bortolozzi:** None.

Poster

602. Rodent Models and Therapeutics in Parkinson's Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 602.04/T13

Topic: C.03. Parkinson’s Disease

Support: CONACYT Grant #166638 (JA)

FINNOVA grant #224222 (DMF)

Title: Pramipexole combined with transfection of the BDNF gene restores motor coordination in a bilateral model of Parkinson disease in the rat.

Authors: *A. N. BENITEZ[1], P. REYNA[1], A. ESPADAS[1], A. SIERRA[1], V. ANAYA[3], B. FLORÁN[1], D. MARTÍNEZ-FONG[2], J. ACEVES[1];
[1]Dept. of Physiol. and Neurosciences, [2]Nanoscience and Nanotechnology Program, CINVESTAV, Mexico city, Mexico; [3]Neuromorphology Laboratory., UNAM, México city, Mexico

Abstract: Pramipexole mediates its therapeutic action on motor deficits in Parkinson's disease mainly via dopamine D3 receptors of the direct and indirect striatofugal pathways. It has also a protective action inducing the expression and secretion of BDNF, which promotes the survival of dopaminergic neurons and enhances dendritic arborization. Here we assessed the immediate and long-term effects of Pramipexole in a bilateral model of PD to test its impact on motor and non-motor behavior. Pramipexole was administered continuously during 4 ½ months via osmotic pumps and the BDNF gene was transfected to the dopamine nigral neurons using a non-viral transfection method (Gonzalez-Barrios et al., 2006). After one month of the 6-OHDA lesion, we tested the acute effect Pramipexole (1 mg/Kg). It restored locomotor and ambulatory activity, walking speed and motor performance evaluated by the rotarod and beam tests, thus showing that Pramipexole controls the motor deficits induced by the bilateral lesion. Since the therapeutic efficacy of Pramipexole eventually wears off, we evaluated the effect of its chronical and continuous administration combined with the non-viral BDNF gene transfection into the remaining nigral neurons, to assess whether it could modify the course of the parkinsonian condition. The combined treatment restored the motor performance, recovering the crossing time in the beam test and the overall rotarod performance to values similar to those of control normal rats. Untreated animals showed none recovery. The motor recovery was observed starting at 1.5 months of the combined treatment and lasted even 2 months after Pramipexole withdrawal. The bilateral lesioned rats showed a permanent reduced ambulation in the open-field compared with normal rats. The combined treatment did not recovered the ambulation to normal. Since the open-field test evaluates anxiety in a novel environment, these results suggested that the combined treatment did not control the anxiety associated with the dopamine lesion. This assumption was confirmed using the burying test to assess anxiety. The test revealed that the bilateral lesion significantly increased the burying time and that the combined treatment did not reduced the time to normal, indicating that it did not control the anxiety produced by the lesion. Diazepam reversed the anxiety of the treated group but not that of the untreated one. Results suggest that the combined treatment induces plastic changes that result in a permanent recovery of motor behavior, but it did not control the anxiety associated with the dopaminergic depletion.

Disclosures: **A.N. Benitez:** None. **P. Reyna:** None. **A. Espadas:** None. **A. Sierra:** None. **V. Anaya:** None. **B. Florán:** None. **D. Martínez-Fong:** None. **J. Aceves:** None.

Poster

602. Rodent Models and Therapeutics in Parkinson's Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 602.05/T14

Topic: C.03. Parkinson's Disease

Title: A PET study with [11-C] raclopride in Hemiparkinsonism model: preliminary results on the effect of a matriz of TiO_2 DA implanted in the caudate nucleus.

Authors: *A. MIRELES-MONZON, JR[1], P. VERGARA ARAGÓN[2], G. VALVERDE AGUILAR[3], H. GONZALEZ SANCHEZ[2], M. PALOMERO RIVERO[4], R. GONZALEZ TREJO[5];
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Abstract: It is now widely accepted that compensatory mechanisms are involved during the early phase of Parkinson's disease (PD) to delay the expression of motor symptoms. However, the neurochemical mechanisms underlying this presymptomatic period are still unclear. Objective: Determine the effects that an implant TiO2DA inserted in the caudate in a rat model hemiparkinsonism on motor behavior and its correlation with the in vivo binding of [11-C] raclopride to D2 dopamine receptors in the basal ganglia of rats with hemiparkinsonism. Each rat underwent a PET study, before and after of treatment with microimplant. Material and methods: Male Wistar rats (250-300gr) were used, which were randomly divided into 4 groups: a) Sham b) Lesioned (Lx); c) Lx+implant; d) Implant. Post-lesion for 21 days anxiety behavior and locomotor activity of the rats of each group through the open field test was evaluated. The test was conducted in an acrylic box (with transparent walls and floor), whose floor is divided with painted black lines forming's squares and illuminated with floodlights. The test was recorded for five minutes; the following measurement parameters were assessed: total distance traveled and the number of crossed lines marked on the floor. The tests were recorded. In each group analysis of microPET was done, each rat underwent a PET study, before and after of treatment with microimplant. Results: The implant induced an increase in the in vivo binding of [11C] raclopride in the striatum of hemiparkinsonian rats. This observation indicates that there is a higher amount of transporters bound to striatal dopamine; higher dopamine levels were found in the Lx+Imp group than in the Lx group as well as a larger number of dopaminergic neurons in striatum in the histological analysis. Conclusions: This observation indicates that in hemiparkinsonian rats, the microimplant with dopamine produce an increase in extracellular

levels of dopamine sufficiently to inhibit raclopride binding, this effect probably due to dopamine release from TiO2DA matrix implanted in caudate nucleus.

Disclosures: **A. Mireles-monzon:** None. **P. Vergara Aragón:** None. **G. Valverde Aguilar:** None. **H. Gonzalez Sanchez:** None. **M. Palomero Rivero:** None. **R. Gonzalez Trejo:** None.

Poster

602. Rodent Models and Therapeutics in Parkinson's Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 602.06/T15

Topic: C.03. Parkinson's Disease

Title: Addressing limb use asymmetry in the unilaterally dopamin depleted parkinsonian rat by combining optogenetics and microdialysis in freely-moving animals

Authors: ***P. K. BHUPAL**[1], J. LYNN[2], J. TURNBULL[1], J. HOVATER[1], K. ANDERSON[1], T. ARVOY[1], K. HOOLSEMA[1], T. LEDY[1], T. TIBBE[1], K. NICHOLSON[1], E. BURKETT[1], M. SANDSTROM[1];
[1]Neurosci., Central Michigan Univ., MT Pleasant, MI; [2]Wayne State, Detriot, MI

Abstract: Optogenetics is an emerging tool in neuroscience that can provide temporally accurate neuronal stimulation of select populations of neurons and to date the large majority of experiments have used this in the context of single-unit electrophysiology. Yet an emerging concern is that the phasic control of dopamine (DA) release in the context of replacement therapy for Parkinson's disease may provide both improved movement control and prolonged therapeutic success. Our laboratory has been designing a way to address the value of phasic DA release within the striatum of otherwise unilaterally DA-depleted rats. To accomplish this we will combine the use of optogenetics with light-sensitive DA-releasing cell transplants and microdialysis for DA measurement in freely moving rats. Initially such DA source cells will be generated from mesenchymal stem cells and transfected with channelrhodopsin II, and our capacity to stimulate controlled levels of DA from these cells will be tested in-vitro. Subsequently such DA source cells will be transplanted into the striatum of a unilaterally DA-depleted rat in order to demonstrate light-stimulated-DA-release-related behavioral symmetry restoration. There are two ways to demonstrate the value of phasic release in the context of replacement therapy for Parkinson's disease. One would be to show that standard basic tonic release is insufficient to restore controlled behavior, and the other would be to show that induced phasic release engaged in an *as-needed* manner is capable of supporting complex movement behaviors. Our technique should be able to address both of these concerns.

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Poster

602. Rodent Models and Therapeutics in Parkinson's Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 602.07/T16

Topic: C.03. Parkinson's Disease

Title: The neuroprotective potential of intranasal DNSP-11 in an intrastriatal 6-hydroxydopamine rat model: a behavior and cellular study

Authors: ***C. M. FOX**[1], M. SPENCER, 18018[2];
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Abstract: Using both *in vitro* and *in vivo* studies, glial cell line-derived neurotrophic factor (GDNF) became known as one of the more promising neurotrophic factors in its ability to protect dopamine neurons against neurotoxic insult in animal models of Parkinson's disease (PD). The proprotein version of GDNF has been post-translationally processed into a dopamine neuron stimulating peptide, known as DNSP-11. DNSP-11 has been shown to be neuroprotective against TaClo, MPP+ and an intranigral 6-hydroxydopamine (6-OHDA) lesion in rat models of PD. This research project used a different approach to introducing DNSP-11 into the animal model prior to the lesion. An intranasal DNSP-11 technique was used to assess the protection of nigral dopamine neurons against the more progressive intrastriatal lesion of 6-OHDA. Twenty Fisher 344 rats were divided into the following groups: citrate + 6-OHDA and DNSP-11 + 6-OHDA. Citrate or DNSP-11was delivered intranasally 5 days per week for 8 weeks postlesion. The foot fault and cylinder tests were performed to assess behavior improvements following treatment. Brain tissue was processed for tyrosine hydroxylase immunocytochemistry and dopamine cell survival in the substantia nigra was quantified.

Disclosures: **C.M. Fox:** None. **M. Spencer:** None.

Poster

602. Rodent Models and Therapeutics in Parkinson's Disease

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Program#/Poster#: 602.08/T17

Topic: C.03. Parkinson's Disease

Support: Faculty of Medicine Universidad Nacional Autónoma de México Project: 080 2012

SIP project: 20160469

Title: Skilled reaching and brain histological evaluation of hermiparkinsonian rats after dopamine supply restitution through a TiO_2DA implant placed in striatum

Authors: *R. MAYEN DÍAZ[1], G. VALVERDE AGUILAR[2], D. VÁZQUEZ MATÍAS[3], H. GONZÁLEZ SÁNCHEZ[4], V. ACEVES SIERRA[4], R. GONZALEZ TREJO[4], P. VERGARA ARAGON[5];
[1]Univ. Nacional Autónoma De México, Ciudad DE Mexico, Mexico; [2]CICATA Legaria Inst. Politecnico Nacional, Mexico City, Mexico; [3]Fisiología, Facultad de Medicina, Univ. Nacional Autónoma de México, Coyoacán, Mexico; [5]Fisiología, [4]Facultad de Medicina, Univ. Nacional Autónoma de México, Coyoacan, Mexico City, Mexico

Abstract: The aim of this study was to assess the effect produced by the placement of a dopamine releasing reservoir in the striatum of hemiparkinsonian rats on fine motor performance, and describe the histological findigs in striatum after 180 days of treatment. Methods: 50 male Wistar rats divided in four groups: A) 8 Sham rats, B) 12 hemiparkinsonian rats induced by administration of 6-OHDA (8µg/4µl) via stereoscopic surgery in in left substantia nigra (Lx), C) 20 hemiparkinsonian rats induced alike Lx rats, and later placement of a dopamine reservoir made of titanium dioxide (Lx+TiO2DA) and D) 10 rats only with placement of the dopamine reservoir (TiO2DA). 21 days after hemiparkinsonism induction in the groups Lx and Lx+TiO2DA, all the groups underwent to apomorphine induced rotational test to evaluate the damage in the dopaminergic system. Additionally, after 180 days of dopamine reservoir placement, skilled reaching was measured using the Eshkol - Wachman scale. Once finalized the evaluations, animals were slaughtered to obtain the brains and the neuron density was analyzed by optical microscopy. Data was analyzed by one-way ANOVA and multiple comparisons Tukey´s test; p values < 0.001 were considered significant. Results: Apomorphine rotational behavior showed an increased number of spins in Lx compared to Sham, Lx+TiO2DA and TiO2DA Groups: 295.2 ± 10.1 vs 4.4 ± 0.8, 30.1±4.2 and 11.0±2.9. Lx+TiO2DA and TiO2DA do not present differences compared to the Sham Group. Measurement of successfully reached pellet attempts revealed a diminishment of reached pellets in Lx animals compared to Sham, Lx+TiO2DA and TiO2DA groups: 8.0±0.4 vs 16.1±0.6, 12.1±0.6 and 13.75±0.6. Lx+TiO2DA animals didn't show differences in skilled reaching task either with Sham or TiO2DA groups.

Histological analysis revealed significant changes in the neuronal populations in striatum where Lx group showed less population than Sham, Lx+TiO2DA and TiO2DA Groups; as well as, when the location of the reservoir was analyzed, it didn't display histopathological data suggesting chronic inflammatory changes, such as cellular edema, gliosis or mononuclear infiltrate in the tissue surrounding the reservoir. Conclusion: Behavioral results in rotational and skilled reaching demonstrate the improvement produced by the dopamine reservoir in Lx+TiO2DA animals reducing the number of spins and increasing the Eshkol-Wachman score compared to Lx animals who displayed a poor performance during both test. Histological analysis demonstrate that the reservoir do not present significant tissue alterations.

Disclosures: **R. Mayen Díaz:** None. **G. Valverde Aguilar:** None. **D. Vázquez Matías:** None. **H. González Sánchez:** None. **V. Aceves Sierra:** None. **R. Gonzalez Trejo:** None. **P. Vergara Aragon:** None.

Poster

602. Rodent Models and Therapeutics in Parkinson's Disease

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Program#/Poster#: 602.09/T18

Topic: C.03. Parkinson's Disease

Support: NMRC TCR14/031

Title: Umbilical cord lining-derived induced pluripotent stem cells as a source for cell replacement therapy of Parkinson's Disease

Authors: *C. CHAI[1], R. Y. ONG[1], C. W. ZHANG[1], B. H. CHAI[1], L. QIU[2], M. D. THANGAVELOO[3], D. YU[5], T. W. SOONG[5], C. S. L. TANG[3,7,8], B. T. ANG[3,5,7,8,4], L. ZENG[2,8], Z. WANG[9], B. GULYÁS[9], T. T. PHAN[6], K. L. LIM[1,8];
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Abstract: Cell replacement therapy holds tremendous promise for the treatment of neurodegenerative diseases such as Parkinson's Disease (PD). However, the lack of a reliable and suitable source of donor cells has limited the widespread application of this treatment modality in the clinic. The advent of induced pluripotent stem cell (iPS) technology has opened up the

possibility for autologous cell therapy. Based on the knowledge that iPS can inherit certain properties of their parental somatic cells via epigenetic transmission, we postulate that deriving iPS from tissues that inherently display reduced immunogenicity and that are genetically pristine could generate an ideal universal donor cell for allogeneic transplantation. The human umbilical cord is an immuno-privileged organ that mediates interactions across the feto-maternal interface. Additionally, its relative nascence implies that it accumulates less somatic mutations compared to adult cells. Capitalizing on these beneficial properties, we derived transgene integration- and feeder-free iPS from cells isolated from the subamniotic lining of the human umbilical cord. Designated as Cord Lining iPS (CLiPS), these iPS showed robust trilineage differentiation either by teratoma formation or directed differentiation in vitro. We further demonstrated that CLiPS can be directed to differentiate into dopaminergic (DA) neurons, the specific neuronal subtypes lost in PD. Transplantation of neuroprogenitor cells (NPCs) primed towards DA fate into the brains of 6-hydroxydopamine induced mouse PD model suggests that these cells can robustly engraft and survive for up to a month in an immunocompetent C57/BL6 mice in the absence of pharmacologic immunosuppression. Furthermore, these NPCs showed efficient differentiation into tyrosine hydroxylase immunoreactive DA neurons. We showed using [18F]FE-PE2I PET imaging that CLiPS-derived NPCs restored dopamine reuptake function in lesioned brains 6 months after transplantation. Behavioral assays revealed statistically significant recovery of motor deficits in transplanted mice. In contrast, no engraftment or behavioral recovery was observed in mice transplanted with human adult iPS-derived NPCs. In summary, we provide proof-of-concept evidence that CLiPS can be used as a source of donor cells for allogeneic cell replacement therapy of PD and potentially other neurodegenerative disorders.

Disclosures: **C. Chai:** None. **R.Y. Ong:** None. **C.W. Zhang:** None. **B.H. Chai:** None. **L. Qiu:** None. **M.D. Thangaveloo:** None. **D. Yu:** None. **T.W. Soong:** None. **C.S.L. Tang:** None. **B.T. Ang:** None. **L. Zeng:** None. **Z. Wang:** None. **B. Gulyás:** None. **T.T. Phan:** Other; CellResearch Corporation Pte Ltd. **K.L. Lim:** None.

Poster

602. Rodent Models and Therapeutics in Parkinson's Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 602.10/U1

Topic: C.03. Parkinson’s Disease

Support: MJFF

Title: Optogenetic inhibition of STN rescues motor defects in parkinsonian rodents.

Authors: *T. NGUYEN-VU[1], L. LEUNG[2], A. ALLAWALA[2], C. ARNOLD[2], D. ZWILLING[2], R. SAWATZKI[2], M. KAPLITT[2];
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Abstract: Parkinson's disease is a motor neurodegenerative disorder characterized at least in part by hyperactivity of the subthalamic nucleus (STN). Although deep brain stimulation (DBS) targeted to the STN is a therapeutic option, its underlying mechanism continues to be a subject of debate. While DBS mimics the effects of destructive lesioning, there is also evidence to support the belief that DBS changes firing patterns, or even drives STN firing. Certain DBS effects have also been ascribed to stimulation outside of the STN in the zone incerta. Using optogenetics, we tested whether functional lesion of the STN can provide therapeutic rescue in 6-OHDA induced hemi-parkinsonian rodents. We confirmed functional inhibition of STN by electrophysiological optrode recording in anesthetized and awake head-fixed rodents. In freely moving hemi-parkinsonian rodents, we were able to show therapeutic rescue of motor function by optogenetic inhibition of the STN. The efficacy of this motor rescue was titratable, in that it was dependent on the intensity of the light and was apparent through multiple behavioral assays. Optogenetic inhibition of the STN in 6-OHDA lesioned rodents attenuated the pathological ipsilateral rotation evoked by amphetamine treatment, and induced contralateral rotation in the absence of any drug treatment. In the same hemi-parkinsonian rodents, light treatment also increased motor activity in an open field assay, improved gait in the catwalk assay, and normalized forelimb preference in the cylinder assay. These results support STN inhibition as a potential therapeutic strategy for treatment of Parkinson's disease.

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Poster

602. Rodent Models and Therapeutics in Parkinson's Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 602.11/U2

Topic: C.03. Parkinson's Disease

Title: A dopamine release from microimplant with nanopores and its effects on neuron density and behavior in a hemiparkinsonian rat model.

Authors: *R. GONZALEZ-TREJO[1], S. HERNANDEZ CASTRO[2], V. E. ACEVES SIERRA[2], G. VALVERDE AGUILAR[3], P. VERGARA ARAGÓN[2];
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Abstract: The purpose for this study was to evaluate the effects produced by a TiO2 microimplant made with nanoporos able to release dopamine molecules, which is placed in caudate nucleus, over motor impairment and neuronal density in Substantia Nigra, and determine whether there is a correlation between neuronal density and gyrus behavior test. Materials and Methods: 40 male Wistar rats divided in 4 groups were utilized: A) 10 control rats: (Control), B) 10 injured rats by 6-OHDA 8μg/4μl through stereoscopic surgery in left and right median forebrain bundle: (Lx), C) 10 rats with the same lesion and the colocation of a micro-implant made of titanium dioxide: (Lx+TiO2DA), and D) 10 rats only with the colocation of micro-implant: (TiO2DA). 21 days after the injury all groups were underwent to the rotational induced test with apomorphine (0.05 mg/kg, sc) during 50 minutes in order to determine damage in dopaminergic system, this test was performed again 360 days after the colocation of the micro-implant. Once finalized the test, the animals were slaughtered to obtain the brains and the neuron density was analyzed by optical microscopy. p values <0.0001 were considered significant. Results: In the rotation induced test, the following results were found at 360 day: the Lx group showed more twists than control, Lx + TiO2DA and TiO2DA groups: control vs Lx (4.4±2.6 vs 301.3±19.0), control vs Lx+TiO2DA (4.4±2.6 vs 31.8±14.9), Lx vs Lx+TiO2DA (301.3±19.0 vs 31.8±14.9), Lx vs TiO2DA (301.3±19.0 vs 9.5±7.1), and Lx+TiO2DA vs TiO2DA (31.8±14.9 vs 9.5±7.1). Analysis of neuronal density in left Substantia Nigra were found 5 significant results, where groups with implants and control had a higher neuronal density than injured groups: Control vs Lx (8.3 ± 1.5 vs 0.5 ± 0.5), control vs Lx + TiO2DA (8.3 ± 1.5 vs 5.7 ± 1.0) , Lx vs Lx + TiO2DA (0.5 ± 0.5 vs 5.7 ± 1.0), TiO2DA vs Lx (0.5 ± 0.5 vs 9.5 ± 1.5), Lx + TiO2DA vs TiO2DA (5.7 ± 1.0 vs 9.5 ± 1.5). On the right side were found 3 significant results where the implanted and control groups had a higher neuronal density than injured groups: Control vs Lx (8.8 ± 1.2 vs 4.8 ± 0.8), Lx vs Lx + TiO2DA (4.8 ± 0.8 vs 7.8 ± 1.5) and Lx vs TiO2DA (4.8 ± 0.8 vs 9.6 ± 3.0). The Pearson correlation between neurons of both left and right Substantia Nigra in the induced rotational behavior showed a negative association with coefficient: r = -0.9458; r = -0.9641 for each case. Conclusion: The present results indicate that microimplant gets reverse engines changes produced by the lesion with 6-OHDA in rats with hemiparkinsonism, as observed in the Lx + TiO2DA group which decreased their number of rotations, likewise the Lx + TiO2DA rats showed a higher neuronal density than Lx rats.

Disclosures: R. Gonzalez-Trejo: None. **S. Hernandez Castro:** None. **V.E. Aceves Sierra:** None. **G. Valverde Aguilar:** None. **P. Vergara Aragón:** None.

Poster

602. Rodent Models and Therapeutics in Parkinson's Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 602.12/U3

Topic: C.03. Parkinson's Disease

Title: TiO_2DA micro-implant can reverse the motor skill alterations caused by 6-OHDA in the reaching for single pellet in a hemiparkinson rat model.

Authors: *P. VERGARA-ARAGON[1], G. VALVERDE AGUILAR[2], R. GONZALEZ TREJO[3], H. GONZALEZ SANCHEZ[4], M. PALOMERO RIVERO[5];
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Abstract: Introduction: Neurodegenerative diseases such as Parkinson's can cause severe motor deficits in skilled forelimb use in both humans and rats. These deficits are typically analyzed in a reach-to-eat paradigm. Therefore, rats serve as an excellent tool to monitor the development of deficits after neurological insults and the single pellet reaching test is a paradigm that involves detailed rating and analysis of qualitative aspects of the reaching movement itself. Objective: The purpose of this research was to evaluate the temporal stability of dopamine in a titanium dioxide matrix (TiO2/DA) in vivo and in vitro and to determine if this micro-implant TiO2DA/DA can reverse motor skill alterations caused by 6-OHDA in the reaching for single pellet in a hemiparkinson rat model. Material and Methods: Sixty four Wistar rats were used, divided into 4 groups: Sham, Lx (hemiparkinsonian), Lx + micro-implant, and micro-implant only. Induced rotation test and single-pellet reaching task were performed on days 0, 1, 21, 90, 180 and 360 days after the insertion of the micro-implant in caudate nucleus. Previously the states of dopamine oxidation were evaluated using infrared spectroscopy. Results: It was possible to evidence stabilization of dopamine molecules 720 hours in vitro and at least during the 360 days that the implant remained in the rat's brain. Significant differences were shown between the Lx group and the control in all the applied tests. Significant differences were also found between the Lx and the Lx+Imp groups, with reports of improvement in the implanted group; Less induced rotations and better performance in the fine motricity assessment (reaching task).These results suggest that probably dopamine was released from its reservoir in the striatum; evidenced by a significant gross motor skill improvement during the 360 days of the experimental evaluation. This motor skill improvement was associated with the presence of dopamine coming from the dopaminergic that was successfully stabilized. Conclusion: Microimplant placement (TiO2DA) in hemiparkinsonian rats improved fine motor skill task, probably due increased levels of dopamine in caudate nucleus. These findings are promising for

the use of these micro-implants as alternative treatment for humans with Parkinson's disease in the near future. Advantages of this treatment are its easy and fast elaboration, microscopic size, low cost, less invasive application, and immediate benefits on the motor disturbances presented by the animals with hemiparkinsonism.

Disclosures: P. Vergara-Aragon: None. **G. Valverde Aguilar:** None. **R. Gonzalez Trejo:** None. **H. Gonzalez Sanchez:** None. **M. Palomero Rivero:** None.

Poster

602. Rodent Models and Therapeutics in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: CNPq

CAPES

FAPESP

NAPNA-USP

Title: Evaluation of the effects of the treatment with gm1 ganglioside in a model of parkinsons disease in rats

Authors: *D. A. SANTOS, III[1,2], P. OLIVEIRA[2], L. BRITTO[2];
[1]Inst. of Biomed. Sci., Univerty São Paulo, São Paulo, Brazil; [2]Univ. São Paulo, São Paulo, Brazil

Abstract: Several evidences have featured the beneficial effects of the GM1 ganglioside in the treatment of neurological lesions. GM1 has also been studied for treatment of neurodegenerative diseases, mainly because of its neuroplastic and neuroprotective functions. The aim of this study was to analyze the possible effects of GM1 ganglioside on the Parkinson's disease model induced by unilateral intra-striatal injection of 6-hydroxydopamine (6-OHDA). Wistar rats (3 months old, approx. 300g) were divided into 3 groups (5-6 animals): control, 6-OHDA, and 6-OHDA + intracerebroventricular (ICV) GM1. In order to promote the lesion, 6 µg/µL of 6-OHDA were stereotaxically administered by microinfusion in the striatum, with two injections of 0.5 µL at 2 different coordinates. GM1 (30 mg /kg dosis) was injected ICV together with 6-OHDA. Seven days after injury, brains were collected for analysis by immunoblotting in the midbrain and striatum. The expression of tyrosine hydroxylase (TH) in the striatum significantly decreased in

group 6-OHDA compared to controls (50.7%, $p<0.01$). The group treated ICV with GM1 showed a significant increase compared to the 6-OHDA group (51.6%, $p<0.01$), recovering the TH expression to the control level. In the midbrain TH expression also decreased significantly in the 6-OHDA group (ca. 51.8%, $p<0.05$) and appeared to be recovered by GM1. Glial fibrillary acidic protein (GFAP) expression in the striatum showed an increase after 6-OHDA (57.9%, $p<0.01$), and showed a decrease in the group treated with GM1 when compared to the group 6-OHDA (36.3%, $p<0.01$). Similarly, in the midbrain GFAP expression showed an increase after 6-OHDA compared with the control (68.0%, $p<0.01$), and the GM1 treated group showed a significant decrease compared with the 6-OHDA group (36.2%, $p<0.05$). Caspase-3 expression in the striatum significantly increased in the 6-OHDA group compared to controls (70.8%, $p<0.01$) and significantly decreased in the group treated with GM1 compared to group 6-OHDA (45.1%, $p<0.05$). In the midbrain there was an increase of caspase-3 expression in the 6-OHDA group (65.6%, $p<0.05$), which was unaltered by treatment with GM1. We suggest that the GM1 ganglioside exerts neuroprotection in the 6-OHDA model of Parkinson´s disease when injected ICV.

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Poster

602. Rodent Models and Therapeutics in Parkinson's Disease

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Program#/Poster#: 602.14/U5

Topic: C.03. Parkinson’s Disease

Support: Carver College of Medicine

Title: Virally-mediated RNA-interference of alpha-synuclein is well-tolerated and effective in animal models of PD

Authors: Y. KIM[1], A. MILLER[1], L. LINS[1], M. KEISER[2], R. BOUDREAU[1], B. DAVIDSON[2], *N. S. NARAYANAN[1];
[1]Neurol., Univ. of Iowa Roy J and Lucille A Carver Col. of Med., Iowa City, IA; [2]Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Parkinson’s disease is a neurodegenerative disease that has no disease-modifying therapy. Alpha-synuclein is postulated to play a key role in disease pathogenesis. Aggregation of the protein alpha-synuclein in insoluble fibrils leading to cellular toxicity in dopamine neurons in humans and in animal models of PD. Here, we use virally-mediated RNA-interference to selectively and specifically reduce alpha-synuclein expression and demonstrate tolerability and

neuroprotection. Previous similar approaches however also induced inflammation and reduced expression of tyrosine hydroxylase. Here we focused on the toxicity due to potential off targeting effect. First, we used a specificity-focused siRNA design algorithm, siSPOTR to identify 8 siRNA sequences with minimal off-targeting potential. After testing these sequences *in vitro*, one RNA-interference sequence (miSyn4) showed maximal protein knockdown potential. To test specificity *in vivo*, AAV vector with miSyn4 was designed and injected in the substantia nigra of mice and rats. miSyn4 was robustly expressed and did not detectably change neurochemistry, dopamine neurons, glial proliferation or animal behavior. We injected AAV-miSyn4 into *Thy1-hSNCA* mice over expressing alpha-synuclein and found significant alpha-synuclein RNA knockdown in both midbrain and cortex. Co-injection of AAV-hSNCA and AAV-miSyn4 demonstrated effective reduction of hSNCA and rescue of SNCA-mediated behavioral deficits. Together, downregulation of SNCA based on RNA interference has therapeutic potential and could be safe and effective for patients with Parkinson's disease.

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Poster

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Program#/Poster#: 602.15/U6

Topic: C.03. Parkinson's Disease

Support: Eurostars program ES#5553

FCT fellowship SFRH/BD/78077/2011

Title: Zeb013 rescues motor impairments in a mouse model of levodopa-induced dyskinesia

Authors: *R. L. VAZ[1], D. CHAPELA[1], J. E. COELHO[2], L. V. LOPES[2], N. D. AFONSO[1], T. F. OUTEIRO[3], S. SOUSA[1];
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Abstract: Parkinson's disease (PD), the second most common neurodegenerative disease, is known for cardinal motor symptoms that include bradykinesia, resting tremor, muscular rigidity and postural instability. Levodopa (L-dopa) is a powerful treatment for the motor symptoms in PD. However, its effectiveness decreases over time and the non-responsive periods to this therapeutic approach increase in advanced stages of the disease. The lack of alternative

therapeutic options for these periods, together with an aggravation of the symptoms is major concern. Novel animal models that recapitulate this advanced stage of the disease are needed in order to enable the development of new therapeutic strategies. Here, we report Zeb013 as a new alternative therapeutic option for PD. Zeb013 was selected in an *in vivo* screen using a 6-hydroxydopamine zebrafish model that enables the identification of compounds rescuing locomotor deficits. We then developed a rodent model that mimics the non-responsive periods of L-Dopa, so that the selected compound could be further investigated in a pre-clinical model. When chronically treated with L-Dopa, this mouse model exhibited an aggravation of gait impairments, and Zeb013 was able to rescue this condition. In the future, we will further investigate the mechanism of action of Zeb013, and gain insight into the molecular underpinnings of advanced stages of PD.

Disclosures: **R.L. Vaz:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Technophage SA. **D. Chapela:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Technophage SA. **J.E. Coelho:** None. **L.V. Lopes:** None. **N.D. Afonso:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Technophage SA. **T.F. Outeiro:** None. **S. Sousa:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Technophage SA.

Poster

602. Rodent Models and Therapeutics in Parkinson's Disease

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

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Topic: C.03. Parkinson’s Disease

Support: Cure Parkinson Trust UK project grant

Title: A novel dual GLP-1 GIP analogue protects the brain in the MPTP mouse model of Parkinson's disease

Authors: *C. HOLSCHER[1], C. JI, Jr.[2], G. XUE, Jr.[2], G. LI[2];
[1]Lancaster Univ., Lancaster, United Kingdom; [2]Neurolog. hospital, Shanxi Med. Univ., Taiyuan, China

Abstract: Glucagon-like peptide 1 (GLP-1) is a growth factor that shows neuroprotective properties. We have shown previously that the GLP-1 mimetics that are currently on the market

as treatments for type II diabetes show neuroprotective effects in animal models of Parkinson's disease (PD). Recently, the GLP-1 mimetic exendin-4 (Bydureon) has shown remarkable effects in a clinical study of PD patients, demonstrating the potential of these drugs. The sister incretin growth factor Glucose-dependent insulinotropic polypeptide (GIP) also has shown protective effects in PD animal models. Here we show that novel dual agonists that activate both GLP-1 and GIP receptors protect the brain in the MPTP mouse model of PD. MPTP was injected once-daily (20mg/kg i.p.) for 7 days, and the dual agonist was once-daily for 14 days (50nmol/kg i.p.). The novel dual agonist reduced or reversed most of the MPTP induced motor impairments in the rotarod, catalepsy, and in a muscle strength test. The number of tyrosine hydroxylase (TH) positive neurons in the substantia nigra (SN) was reduced by MPTP and increased by the drug. The ratio of anti-inflammatory Bcl-2 to pro-inflammatory BAX signaling as well as the activation of the growth factor kinase Akt was reduced by MPTP and reversed by the new drug. The PI3k inhibitor had only limited effect on the drug effect. Importantly, levels of the neuroprotective brain derived neurotropic factor (BDNF) were reduced by MPTP and enhanced after drug treatment. The activation of a chronic inflammation response in the brain by MPTP was much reduced by the drug (astroglia and microglia activation). The results show superior effects over single GLP-1 analogues and suggest that this new dual agonist drug class is more protective in PD than exendin-4 in the clinic.

Disclosures: **C. Holscher:** 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; funded by the Cure Parkinson's Trust UK. **C. Ji:** None. **G. Xue:** None. **G. Li:** None.

Poster

602. Rodent Models and Therapeutics in Parkinson's Disease

Location: Halls B-H

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Program#/Poster#: 602.17/U8

Topic: E.03. Basal Ganglia

Support: Intramural Research Program, NINDS, NIH

Title: Beta and gamma oscillatory activity in the subthalamic nucleus, anterior cingulate cortex, and ventral medial prefrontal cortex in the awake hemiparkinsonian rat

Authors: *A. R. WEISS, C. DELAVILLE, K. B. DUPRE, E. BRAZHNIK, N. NOVIKOV, J. R. WALTERS;
Neurophysiological Pharmacol. Section, NINDS, Bethesda, MD

Abstract: Exaggerated beta oscillatory activity is observed in the motor circuits of Parkinson's disease (PD) patients and animal models of PD. This activity has been hypothesized to contribute to motor dysfunction in PD. We have previously observed significant increases in spectral power in the 25-40 Hz high beta range in local field potentials (LFP) recorded in the subthalamic nucleus (STN), substantia nigra pars reticulata, and motor cortex of parkinsonian rodents associated with motor function. Further, it has been hypothesized that deep brain stimulation and L-DOPA medication reduce motor and non-motor symptoms by disrupting this excessive beta oscillatory activity. However, the extent to which non-motor networks contribute to this high beta activity and the impact of pathological oscillatory activity in influencing the cognitive deficits of PD are not well understood.
This study used 6-OHDA-lesioned, hemiparkinsonian rats performing a circular treadmill walking task to compare synchronized STN LFP activity with activity in the ventral medial prefrontal cortex (mPFC) (i.e., the infralimbic and prelimbic cortices), and the anterior cingulate cortex (ACC, dorsal mPFC), areas involved in cognitive processes. Electrode bundles were implanted in the STN, the ACC, and the ventral mPFC of rats with unilateral dopamine cell lesions. LFP and spiking activity were recorded during epochs of treadmill walking in control animals and 21 days after dopamine cell lesion.
Data show increases in 29-36 Hz high beta LFP spectral power in the STN and ACC and coherence between these regions after dopamine depletion. In contrast, recordings from the ventral mPFC 21 days after lesion did not show peaks in this beta frequency range. Furthermore, ACC, ventral mPFC, and STN showed peaks in the low gamma frequency range in LFP power - the ACC with a mean peak frequency at approximately 57 Hz and the ventral mPFC and STN at 51 Hz - during treadmill walking before and 21 days after dopamine depletion.
These results suggest that the STN integrates activity from mPFC circuits in a manner that varies with behavioral state, oscillatory frequency, and the integrity of the dopamine system. Our results may help to gain further insight into the significance of excessive beta oscillatory activity in PD and its influence on motor and cognitive systems.

Disclosures: **A.R. Weiss:** None. **C. Delaville:** None. **K.B. Dupre:** None. **E. Brazhnik:** None. **N. Novikov:** None. **J.R. Walters:** None.

Poster

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Topic: E.03. Basal Ganglia

Support: Intramural Research Program, NINDS, NIH

Title: Effects of inhibitory DREADD in the substantia nigra pars reticulata on cortical high beta and high gamma LFP oscillations and behavior in hemiparkinsonian rats

Authors: *K. B. DUPRE, C. J. LOBB, H. C. BERMUDEZ CABRERA, J. R. WALTERS, M. A. COHEN;
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Abstract: Loss of dopamine is associated with increased synchronization and oscillatory activity in the basal ganglia in both Parkinson's disease (PD) patients and animal models of PD. Our lab has previously observed increases in LFP power in the 25-40 Hz range in the substantia nigra pars reticulata (SNpr) and motor cortex (MCx) in the hemiparkinsonian rat during treadmill walking, as well as increases in power in the 70-120 Hz range in the MCx during L-DOPA-induced dyskinesia. These findings suggest that the SNpr may play a role in cortical high beta and high gamma LFP activity during bradykinesia and dyskinesia. To study this, hemiparkinsonian rats (n=5) received infusion of the inhibitory DREADD virus, AAV2/8-hSyn-hM4D(Gi)-mCherry, into the SNpr and implantation of electrodes targeting MCx and SNpr. Post-histological analyses revealed DREADD virus expression in the SNpr with some spread to surrounding areas. Similar to our previous work, exaggerated high beta LFP power in the MCx and SNpr and coherence between these regions were observed during treadmill walking. At 3-4 weeks post-surgery, clozapine-N-oxide (CNO; 1.0 mg/kg, i.p.), which stimulates receptors expressed in DREADDs transfected neurons, caused a significant reduction in cortical high beta LFP activity during treadmill walking compared to before CNO. This suggests that inhibiting basal ganglia output may disrupt the propagation of high beta activity observed in the basal ganglia-thalamocortical circuit during bradykinesia. After L-DOPA priming (12 mg/kg, s.c., once daily for 7 days), rats received saline or CNO 15 min prior to L-DOPA (6 mg/kg, s.c.). Similar to our previous findings, cortical high gamma LFP oscillations and abnormal involuntary movements (AIMs) were significantly increased following L-DOPA, regardless of pre-treatment. Interestingly, CNO pre-treatment caused significant increases in both cortical high gamma LFP power and AIMs from approximately 110-160 min post-L-DOPA compared to saline pre-treatment. CNO-mediated inhibition of the SNpr may enhance and extend L-DOPA-mediated inhibition of basal ganglia output, leading to further disinhibition of the thalamus, which would excite the MCx, resulting in exaggerated high gamma activity and dyskinesia. Ongoing DREADDs experiments are further investigating the contribution of the SNpr to cortical high beta and high gamma oscillations during bradykinesia and dyskinesia, as well as the spiking activity of SNpr and MCx under these conditions.

Disclosures: **K.B. Dupre:** None. **C.J. Lobb:** None. **H.C. Bermudez Cabrera:** None. **J.R. Walters:** None. **M.A. Cohen:** None.

Poster

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Support: Magnus Bergvall

Kock

Crafoord

Olle Engkvist

Michael J. Fox

Parkinson Research Foundation

Parkinson Foundation

Title: Changes in neuronal activity of cortico-basal ganglia-thalamic networks induced by dopaminergic manipulations

Authors: *N. IVICA, U. RICHTER, I. BRYS, P. PETERSSON;
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Abstract: The basal ganglia are comprised of several subcortical nuclei that are interconnected with the cerebral cortex, thalamus, and brainstem and are thought to be particularly involved in processes relating to action selection and the execution of goal-directed movements. In the cortico-basal ganglia-thalamic circuitry dopamine plays an important role in maintaining the normal function in movement control. This is evident for example in the symptomatology of Parkinson's disease, where the death of a large fraction of the dopaminergic cells in the midbrain leads to severe motor dysfunctions. In order to investigate how disruptions in dopamine signaling affect the neurophysiological activity in the cortico-basal ganglia-thalamic loop, we have here acquired large-scale neuronal recordings in rodents prior to and following acute and chronic interference with the transmission of this neuromodulator. Acute pharmacological interventions include both the blockage of dopamine synthesis from tyrosine using alpha-methyl-p-tyrosine (AMPT) and antagonism of dopaminergic receptors (either type D1R or D2R, or D1R+D2R). Chronic dopamine depletion was obtained by unilateral medial forebrain bundle 6-OHDA lesions. Neuronal recordings in female Sprague-Dawley rats were obtained simultaneously in 16 different regions of the cortico-basal ganglia-thalamic loop (8 per hemisphere), sampling both local field potentials and single cell activity under the different experimental conditions. In local

field potential recordings we could confirm the previously reported relative increase in power in the beta-band. In particular, the exaggerated beta was found in experiments with chronic depletion as well as with AMPT, D1R+D2R antagonist and D2R antagonist, however, the increase was not as clear for the D1R antagonist alone. At the same time, all acute interventions had very similar behavioral effects, i.e. in all animals drug-induced catalepsy could be confirmed in direct tests. Analysis of unit activity suggested complex patterns of changes in firing rate in the different structures, indicating simple rate models stemming from the concept of opposing effect in the direct and indirect pathway are not sufficient to capture the observed data. These data provide for the first time a detailed description of both the acute and chronic effects of disrupted dopamine signaling on the neurophysiological activity in the cortico-basal ganglia-thalamic loop.

Disclosures: **N. Ivica:** None. **U. Richter:** None. **I. Brys:** None. **P. Petersson:** None.

Poster

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Topic: C.03. Parkinson's Disease

Title: Selective targeting of striatal 5-HT_{1A} auto- or hetero-receptors to alleviate L-DOPA-induced dyskinesia in hemi-parkinsonian rats.

Authors: *S. M. MEADOWS[1], C. TASBER[1], N. E. CHAMBERS[1], M. CONTI[1], E. SHEENA[1], M. A. VARNEY[2], A. NEWMAN-TANCREDI[2], C. BISHOP[1];
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Abstract: L-DOPA is the standard treatment for the akinetic motor symptoms of late-stage Parkinson's disease (PD), however chronic treatment leads to abnormal involuntary movements, known as L-DOPA-induced dyskinesia (LID). Modulation of aberrant striatal dopamine (DA) release from serotonin (5-HT) terminals, via 5-HT_{1A} receptor stimulation, has been shown to attenuate LID severity in rodent and non-human primate models. Clinically, previously-used 5-HT_{1A}R agonists have only been modestly effective at reducing LID and may worsen PD motor symptoms and increase liability for 5-HT syndrome. In recent years, the highly selective G-protein biased 5-HT_{1A}R agonists F13714 and F15599 have been employed to target either pre-synaptic auto-receptors or cortical hetero-receptors, respectively. The present study utilized these compounds to better understand the role of distinct striatal 5-HT_{1A}R populations in dyskinetic, hemi-parkinsonian rats. In Experiment 1, a cohort of rats received systemic injections of vehicle, F13714 (0.01 or 0.02 mg/kg, s.c.), or F15599 (0.06 or 0.12 mg/kg, s.c.) 5 minutes prior to L-

DOPA (6 mg/kg, s.c.) in a within-subjects counterbalanced design. In Experiment 2, a second cohort of rats received bilateral striatal cannulations concurrent with DA lesion. After L-DOPA priming, animals were split into two equally dyskinetic groups, one that received intrastriatal microinjections of F13714 (0, 2 or 10 μg in 1 μL) and another receiving F15599 (0, 10, or 30 μg in 1 μL), 5 minutes prior to systemic L-DOPA (6 mg/kg, s.c.) administration. LID and 5-HT syndrome were rated in both experiments, but motor performance was only rated in Experiment 1. Results from Experiment 1 showed that whilst both compounds effectively reduced dyskinesia without interfering with the therapeutic effects of L-DOPA, stimulation of 5-HT_{1A} autoreceptors induced marked 5-HT syndrome in these acute administration experiments. Striatal microinjections in Experiment 2 yielded a similar pattern of effects, suggesting that these compounds work via striatal 5-HT_{1A}Rs to reduce LID. This study implicates differential roles for striatal 5-HT_{1A} auto- and hetero-receptor populations in the therapeutic effects of non-specific 5-HT_{1A}R agonists on LID expression and suggests the promise of selective 5-HT_{1A}R targeting for clinical LID treatment.

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Poster

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Topic: C.03. Parkinson’s Disease

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Title: Pathological firing patterns in substantia nigra pars reticulata neurons after acute or chronic dopamine deprivation in the 6-OHDA rodent model of Parkinson´s disease

Authors: *V. CACERES CHAVEZ[1], R. HERNÁNDEZ-MARTÍNEZ[2], J. PÉREZ-ORTEGA[1], M. A. HERRERA-VALDÉZ[3], E. GALARRAGA[1], J. BARGAS[1];
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Abstract: During Parkinson's disease, dopamine deprivation produces modifications on several synaptic inputs connecting *substantia nigra pars reticulata* (SNr) neurons (e.g., Ibañez-Sandoval

et al., 2007; Aceves et al., 2011), these changes in turn produce changes in the firing patterns in the neurons of the SNr. This feature may represent the cellular basis of a major pathological sign of the disease in both animal models and patients: correlated firing and anomalous synchrony in basal ganglia output neurons. In the present work, we perform whole cell recordings of SNr neurons in mouse brain slices from healthy and parkinsonian subjects. The firing pattern of SNr neurons in control conditions is mostly regular and tonic. After either acute blockade of D1 and D2 dopamine receptors with their respective selective antagonists (sulpiride and SCH23390) or after the lesion of the dopaminergic neurons with 6-OHDA, the firing pattern of SNr neurons becomes irregular and bursty. Because under control conditions TRP cationic and T type calcium channels have been implicated in the generation of firing of SNr neurons, we decided to determine whether on pathological conditions they remain as an important component of the firing machinery. Here, we confirm that hypothesis. Given that SNr neurons are the first target of the *substantia nigra pars compacta* dopaminergic neurons, we propose that we can assay Parkinson's disease pharmacological treatments evaluating their effects in the firing pattern of these neurons.

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Poster

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Topic: C.03. Parkinson’s Disease

Title: D1R-D3R dopamine receptor stimulation alters downstream signaling in the semi-parkinsonian rat: implications of the D1R-D3R heteromer

Authors: *K. E. LANZA[1], S. MEADOWS[1], M. DEAK[1], C. BISHOP[1], S. FERRÉ[2]; [1]Psychology, Binghamton Univ., Binghamton, NY; [2]Natl. Inst. on Drug Abuse, NIH, Baltimore, MD

Abstract: Parkinson’s Disease (PD) is a neurodegenerative disorder characterized by nigro-striatal dopamine (DA) cell loss resulting in pronounced motor deficits. DA replacement therapy first with DA agonists and subsequently L-DOPA, initially reduces motor symptoms, but eventually patients experience loss of treatment efficacy and abnormal involuntary movements (AIMs) develop. The DA D1 receptor (D1R), expressed on the medium spiny neurons of the direct pathway, has been strongly implicated in dyskinesia and changes in D1R signaling associated with dyskinesia development and expression. DA D3 receptors (D3R) have also been

shown to play a role in dyskinesia development though D3R mechanisms are less understood. More recent work has suggested an interplay between D1R and D3R. In vitro, co-localization of D1R with D3R in the form of a heteromer synergistically enhances select downstream substrates, such as extracellular signal-regulated protein kinase (ERK 1/2) in its phosphorylated form (pERK 1/2). In the unilateral 6-hydroxydopamine rat, D1R and D3R agonists dose-dependently induce dyskinesia and importantly, their co-administration induces synergistic expression of dyskinesia. To better understand how D1R-D3R co-activation leads to in vivo alterations in downstream signaling, we monitored levels of ERK1/2 and pERK1/2 after individual or collective administration of D1R and/or D3R agonists. To do so, adult male Sprague-Dawley rats were given unilateral DA lesions to the medial forebrain bundle. Three weeks later, rats were given daily L-DOPA (6 mg/kg; s.c.) for 2 weeks to establish stable dyskinesia expression. Rats were then treated with either vehicle, the D1R agonist SKF38393, the D3R agonist (+)PD128907 or a combination of both and dyskinetic behaviors were measured. Thirty minutes later, striatal tissue was harvested and later analyzed via western blot. Initial findings indicate pronounced behavioral synergy and treatment-dependent changes in cellular signaling, supporting the induction of pERK1/2 as a cellular consequence of D1R-D3R interactions in the DA-depleted striatum.

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Poster

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Title: Spatiotemporal inhomogeneity of pathological beta activities in the motor cortex of hemi-parkinsonian rats

Authors: *Z. YU[1], W. ASAAD[2,3], A. NURMIKKO[1], I. OZDEN[1];
[1]Sch. of Engin., [2]Neurosci., Brown Univ., Providence, RI; [3]Rhode Island Hosp., Providence, RI

Abstract: Excess beta-band power (13-30Hz beta oscillations) in the local field potentials (LFPs) of the motor cortex and in several basal ganglia nuclei is the most prominent feature of Parkinsonian pathophysiology. However, it is not known how and to what extent pathological beta activity might override the functional activity in the motor cortex. Specifically, the spatiotemporal dynamics of pathological neural activity within the Parkinsonian motor cortical microcircuits have not been characterized at suitable spatial resolution. To address this question, we utilized microelectrode arrays (MEAs, 400μm electrode pitch) implanted unilaterally (6x6 MEA) or bilaterally (two 5x5 MEAs) into the anterior forelimb area of motor cortices in 6-OHDA-induced hemi-Parkinsonian rats. Recordings were obtained during free behavior or while animals performed a harnessed lever pressing task. In short, animals were conditioned to press a lever with their right forelimb upon onset of a light cue to receive a reward. LFPs were extracted from MEA recordings and their spatiotemporal dynamics were examined with spectral, correlation, and coherence analyses. Unilateral 6-OHDA lesions led to stereotypical asymmetrical motor deficits such as akinesia and rotational bias. Accompanying motor deficits, elevated beta-band power in LFPs was observed on the lesioned side of motor cortex. Interestingly, these beta oscillations appeared intermittently only at certain locations as distinct spatial activity patterns. A linear discriminant analysis showed that the beta band power at some recording sites was indistinguishable from control levels. However, further analysis indicated that these sites could be distinguished from control sites by their phase coherence. We found, within the lesioned motor cortex, excess phase coherence at the beta band between pairs of recording sites. The beta synchrony was not distributed uniformly; it was more pronounced between sites with higher beta power. We note that the motor cortex has been tested as an alternative modulation target for Parkinson's disease, e.g. by transcranial magnetic stimulation. In our microcircuit work, the observed variations in beta-band power and phase synchrony across the motor cortex implied the presence of inhomogeneity in the extent of cortical Parkinsonism; and hence, might offer a route to potential therapeutic benefit via differential neuromodulation at different motor cortical sites. Such modulation paradigm could potentially allow more specific control of motor cortical activity, and thereby motor symptoms, while reducing interference of stimulation on eloquent functional motor activity.

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Poster

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Topic: C.03. Parkinson's Disease

Support: F31 NS090745-01

Title: Cell-type specific pallidal stimulation provides long-lasting relief of immobility in a model of Parkinson's disease

Authors: *K. J. MASTRO[1], K. T. ZITELLI[1], A. M. WILLARD[2], K. H. LEBLANC[3], A. V. KRAVITZ[3], A. H. GITTIS[2];
[1]Neurobio., Univ. of Pittsburgh, Pittsburgh, PA; [2]Carnegie Mellon Univ., Pittsburgh, PA; [3]NIDDK, Natl. Institude of Hlth., Bethesda, MD

Abstract: In Parkinson's disease (PD), the external segment of the globus pallidus (GPe) is a key contributor to the induction, propagation and maintenance of network dysfunction within the basal ganglia. In the classical rate model of basal ganglia dysfunction, the loss of dopamine shifts the balance of the two functionally opposing pathways: motor-facilitating direct and motor-suppressing indirect. An overactive indirect pathway leads to the cardinal symptoms of PD: bradykinesia and immobility. To test the efficacy of pallidal stimulation to reduce indirect pathway activity and alleviate parkinsonian motor symptoms, we used an optogenetic approach to modulate activity in the GPe in a global, or cell-selective manner. Our results demonstrate that global increases or decreases in GPe activity are minimally effective at restoring movement in bilaterally dopamine-depleted mice, but in contrast, cell-specific stimulation strategies were highly effective. Specifically, activation of Parvalbumin-positive (PV-GPe) neurons, or inhibition of Lim homeobox 6-positive (Lhx6-GPe) neurons restored movement to near pre-lesion levels. Intriguingly, this behavioral rescue did not cease at the end of stimulation, but persisted for hours. At the end of the 4-hour experiment, all mice still exhibited near pre-lesion levels of locomotion. For comparison, we tested the ability of direct-pathway stimulation to rescue movement in bilaterally-depleted mice. Behavioral recovery as a result of direct pathway stimulation was neither as robust, nor as persistent as cell-specific manipulations in the GPe. In future experiments, we will use in vivo electrophysiology within the output nucleus of the BG to observe circuit-level alterations before, during and after the optogenetic stimulation. In summary, these results demonstrate that cell-specific activation of PV-GPe or inhibition of Lhx6-GPe neurons provide a long-lasting recovery in motor function and establish the existence of two functionally distinct cell populations in the GPe.

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Poster

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Topic: C.03. Parkinson’s Disease

Title: Dopamine depletion alters striatal histone acetylation in the MPTP-induced mouse model of Parkinson's disease.

Authors: *G. L. LEMIEUX, É. PEPIN, G. BUREAU, M. CYR;
Med. Biol., Univ. Du Québec À Trois-Rivières, Trois-Rivière, QC, Canada

Abstract: Parkinson's disease (PD) is a chronic progressive neurodegenerative movement disorder characterized by a profound and selective loss of nigrostriatal dopamine (DA) neurons. Clinical manifestations of this complex disease include motor impairments involving resting tremor, bradykinesia, postural instability, gait difficulty and rigidity. The molecular adaptation of striatal neurons to DA deficiency, mostly accountable for the motor manifestations, remains poorly understood. Epigenetic modifications of histone proteins exert fundamental roles in developmental processes and their deregulation is suspected in the progression of diverse human disorders. Acetylated and deacetylated histones are considered epigenetic tags within chromatin by relaxing or tightening chromatin structure, subsequently increasing or decreasing gene transcription levels. In the present study, we verify whether acetylation of histone proteins play a role in the molecular adaptation of striatal neurons and the impaired motor behavior associated with DA depletion. The neurotoxin MPTP was acutely administered to adult mice using 4 injections of 20 mg/kg, i.p., every 2 hours. Mice were sacrificed 1 or 7 days after MPTP injections. To evaluate motor manifestations, we performed 7 days after treatments the beam, wire suspension and pole tests in vehicle and MPTP-treated mice. Impaired motor abilities were observed in the MPTP-treated mice in all tests; but statistical significance was reached only in the wire suspension test. Degrees of DA-depletion were estimated by the evaluation of tyrosine hydroxylase (TH) levels in the striatum using western blot technique. We observed a 45% and a 60% reduction in striatal TH levels in mice sacrificed 1 and 7 days after MPTP injections, respectively. Modification of histone acetylation was assessed in the striatum of mice following MPTP treatments by western blot. Elevated levels of lysine 9-acetylated histone H3 (H3K9ac) were observed in MPTP mice 1 day after injections. In MPTP-treated mice sacrificed 7 days after injections, levels of H3K9ac were even more important and reached statistical significance. These preliminary results indicate that DA depletion triggers epigenetic modification of histone proteins in the striatum of MPTP-treated mice. Although conclusion about the relationship between PD, motor manifestations and striatal histone acetylation would need further investigations, studying epigenetic mechanisms will be helpful to understand the etiology of PD which is essential for therapeutic strategies of this disease.

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Poster

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Topic: C.03. Parkinson's Disease

Support: NIH Grant 1F31NS093944

Title: Progression of physiological changes in the basal ganglia following bilateral gradual or acute administration of 6-hydroxydopamine in mice

Authors: ***A. M. WILLARD**[1], K. J. MASTRO[2], A. H. GITTIS[1];
[1]Biol. Sci., Carnegie Mellon Univ., Pittsburgh, PA; [2]Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Parkinson's disease (PD) is characterized by the gradual loss of dopamine in the basal ganglia that produces dramatic changes in circuit physiology and ultimately motor function. In humans, levels of dopamine degenerate at a rate of ~11% over the course of 10-20 years where motor deficits do not appear until a significant amount of dopamine is lost. Despite abundant evidence for the gradual loss of dopamine in humans, most studies utilizing neurotoxin ablation of dopamine neurons use acute, unilateral administration to understand PD physiology. Though many of these studies are instrumental in our understanding of basal ganglia circuit dysfunction, the period over which dopamine is lost may be a necessary component for the brain to adapt and therefore more closely recapitulate the physiological characteristics found in the patient population. Based on recent behavioral evidence (Willard et al., 2015), the rate at which dopamine is depleted can impact the degree of motor preservation at late stages. We hypothesized that the gradual decline in dopamine may alter the way in which the basal ganglia adapt to the new brain state and may challenge current descriptions of parkinsonian physiology. In this study, we compared the neural activity within basal ganglia nuclei of awake head-restrained mice that had undergone gradual or acute bilateral dopamine depletion with 6-hydroxydopamine administration in the medial forebrain bundle. Further investigation utilizing gradual bilateral dopamine depletions could aid in uncovering potential compensatory maintenance of neural activity within the basal ganglia, and enhance our understanding of the role of dopamine in basal ganglia function.

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Poster

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Topic: C.03. Parkinson's Disease

Title: Computer Simulation of GBA related pathways with implications for Parkinson 's disease

Authors: *I. IKEDA[1], J. W. RYAN[2], T. J. SWEENEY[3], A. D. LEE[1], B. BEHROUZ[1]; [1]Neuroinitiative, Jacksonville, FL; [2]NeuroInitiative, Jacksonville, FL; [3]Neuriniative, Jacksonville, FL

Abstract: Since the discovery of the link between the glucocerebrosidase (GBA) gene and Parkinson's disease (PD), a substantial amount of attention has been directed towards the mechanisms by which the glucocerebrosidase protein (GCase) and lysosomal dysfunction contribute to α-synuclein pathology and PD. Research into this area has identified a consistent, inverse, and bidirectional relationship between GCase activity and α-synuclein protein concentration, although a specific biochemical relationship has yet to be elucidated. GCase is a lysosomal hydrolase that is active in a dynamic degradative process known as the glycosphingolipid salvage pathway, critical for the recycling and re-synthesis of new lipids. Interestingly, deficient GCase activity results in Gaucher Disease (GD) in an autosomal recessive manner. We used the Simulation Environment for Experimental Design (SEED) to model and simulate, at a pathway level, the molecular interactions of GCase and sphingolipid metabolism. *In Silico* protein content was quantified following various knock out and overexpression models within this pathway and data was visualized and quantified, allowing for the modeling of GCase related abnormalities such as those observed in PD and GD. Additionally the degradative pathway of short chain glycosphingolipids was represented by SEED allowing for the basic modeling of abnormalities in a number of lysosomal storage disorders (e.g GM1 gangliosidosis or Tay Sachs disease).

Disclosures: I. Ikeda: None. **J.W. Ryan:** None. **T.J. Sweeney:** None. **A.D. Lee:** None. **B. Behrouz:** None.

Poster

602. Rodent Models and Therapeutics in Parkinson's Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 602.28/V1

Topic: C.03. Parkinson's Disease

Title: Adressing mitochondrial dysfunction in iPSC derived neurons from GBA1 associated Parkinson's disease patients

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Neurodegeneration, DZNE Tübingen, Tuebingen, Germany

Abstract: Heterozygous mutations in the *β-glucocerebrosidase* (*GBA1*) gene represent the most common genetic risk factor for Parkinson's disease (PD) known to date. Even though mitochondrial dysfunction has been described to play a role in the disease, it is still unclear whether a loss of glucocerebrosidase enzymatic activity or a gain of function of the misfolded enzyme contributes to disease pathogenesis. In order to study the role of *GBA1* loss of function in PD, we generated *GBA1* knock out ($^{-/-}$) induced pluripotent stem cells (iPSCs) using CRISPR–Cas9 technology. To this end, a deletion was introduced in both copies of the *GBA1* gene in iPSCs from healthy individuals as well as PD patients with *GBA1* mutations. Multiple KO lines from each subject were selected and differentiated into midbrain dopaminergic (DA) neurons. No significant differences in DA neuronal differentiation potential were observed between *GBA1*$^{-/-}$ iPSCs and corresponding isogenic controls. *GBA1* PD and *GBA1*$^{-/-}$ neurons showed increased production of reactive oxygen species, impaired respiration and reduced mitochondrial membrane potential compared to wild type isogenic controls. Next, we assessed energy metabolism in iPSC-derived neurons and investigated the therapeutic effect of targeting mitochondrial function in GBA-PD.
Our data suggest that mitochondrial dysfunction plays a role in the pathogenesis of GBA-PD, thereby providing a tool for the development of therapeutic strategies for PD.

Disclosures: D.C. Schöndorf: None. **L.K. Schwarz:** None. **D. Invanyuk:** None. **M. Zarani:** None. **S. De Cicco:** None. **T. Gasser:** None. **M. Deleidi:** None.

Poster

602. Rodent Models and Therapeutics in Parkinson's Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 602.29/V2

Topic: C.03. Parkinson's Disease

Title: Inhibition of glucosylceramide synthase alleviates aberrations in synucleinopathy models

Authors: *S. SARDI, C. VIEL, J. CLARKE, A. RICHARDS, H. PARK, J. DODGE, J. MARSHALL, B. WANG, S. CHENG, L. S. SHIHABUDDIN;
Genzyme, a Sanofi Co., Framingham, MA

Abstract: Mutations in *GBA*, the gene encoding glucocerebrosidase, are associated with an enhanced risk of developing synucleinopathies such as Parkinson's disease (PD). Recent studies have also demonstrated that genetic variation in *GBA* can impact the progression of PD. Patients harboring mutations in *GBA* present higher prevalence and severity of motor and non-motor symptoms. However, the precise mechanisms by which mutations in *GBA* increase PD risk and exacerbate its progression remain unclear. Here, we investigated the merits of glucosylceramide synthase (GCS) inhibition as a potential treatment for synucleinopathies. A Gaucher-related synucleinopathy mouse model ($Gba^{D409V/D409V}$) was treated with an orally available brain-penetrant GCS inhibitor, Genz-667161 for 8 months. This intervention prevented CNS substrate lipid accumulation. Most notably, treatment with the GCS inhibitor slowed the accumulation of hippocampal aggregates of α-synuclein, ubiquitin and tau, and improved the associated memory deficits. The effects of the GCS inhibitor were also studied in a mouse model overexpressing α-synuclein, *PrP-A53T-SNCA,* and harboring wild type alleles of *GBA*. Treatment of *PrP-A53T-SNCA* mice with Genz-667161 for 6.5 months reduced membrane-associated α-synuclein in the CNS and ameliorated cognitive deficits. Collectively, the data indicate that inhibition of GCS can modulate processing of α-synuclein and reduce various α-synuclein entities, thereby reducing the progression of synucleinopathies in mice with and without mutations in *GBA*. The present study provides supporting evidence for the clinical development of brain-penetrant GCS inhibitors in PD and other synucleinopathies.

Disclosures: **S. Sardi:** A. Employment/Salary (full or part-time): Sanofi Genzyme. **C. Viel:** A. Employment/Salary (full or part-time): Sanofi Genzyme. **J. Clarke:** A. Employment/Salary (full or part-time): Sanofi Genzyme. **A. Richards:** A. Employment/Salary (full or part-time): Sanofi Genzyme. **H. Park:** A. Employment/Salary (full or part-time): Sanofi Genzyme. **J. Dodge:** A. Employment/Salary (full or part-time): Sanofi Genzyme. **J. Marshall:** A. Employment/Salary (full or part-time): Sanofi Genzyme. **B. Wang:** A. Employment/Salary (full or part-time): Sanofi Genzyme. **S. Cheng:** A. Employment/Salary (full or part-time): Sanofi Genzyme. **L.S. Shihabuddin:** A. Employment/Salary (full or part-time): Sanofi Genzyme.

Poster

602. Rodent Models and Therapeutics in Parkinson's Disease

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Program#/Poster#: 602.30/V3

Topic: C.03. Parkinson's Disease

Support: NIH R01 NS079312

NIH R37 NS040894

Title: Cortical evoked potentials generated by deep brain stimulation

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Abstract: Parkinson's disease (PD) is associated with abnormal neural activity in the basal ganglia and cortex. Subthalamic nucleus (STN) and globus pallidus (GP) deep brain stimulation (DBS) are both effective in suppressing PD motor symptoms by altering cortical activity, however the anatomical pathways responsible for this modulation remain unclear. Cortical evoked potentials (cEP) generated by STN or GP DBS are a key biomarker encoding the response of cortex to subcortical stimulation. The goal of this study was to determine the neural origin of STN and GP DBS induced cEP. We performed M1 electrocorticography (ECoG) recordings with skull mounted screws during 4.5 Hz STN and GP DBS in healthy awake rats. We then used a detailed biophysical model of the thalamocortical (TC) network (Traub et al. 2005) to decipher the neural origin of the cEP signal.

The predominant in vivo STN DBS induced cEP pattern (n=7/16 rats) included short latency positive (P1), intermediate latency negative (N1), and long latency positive (P2) responses. Major features of the STN DBS induced cEP response pattern in rats matched well with those from humans (Walker et al. 2012), although there were differences in peak latencies between the rat and human cEPs. STN DBS was simulated in the computational model by activating axons of layer 5 pyramidal neurons and by injecting inhibitory postsynaptic current into thalamus (TH) mimicking activation of antidromic (corticospinal, CS) and orthodromic (STN-entopeduncular nucleus-TH) pathways respectively. Model-based STN DBS cEPs matched remarkably well in vivo responses. P1 and N1 responses were due to the direct and rebound activation of L5 pyramidal neurons, respectively, while polysynaptic activations of L5 and L2/3 pyramidal neurons resulted in the long latency response. Orthodromic activation produced an inhibitory effect on TH and reduced the amplitude of the long latency response.

GP DBS induced cEP (n=6/11 rats) exhibited P1, N1, P2 responses similar to STN DBS, however the magnitude and latency of response components were different. GP DBS was simulated in the computational model by stimulating axons of L5 pyramidal and TH neurons

mimicking activation of CS and TC fibers in the internal capsule. P1 response in model cEP was due to the direct activation of L5 pyramidal neurons, while trans-synaptic activations of L2/3 and L6 pyramidal neurons generated N1 and P2 responses.
Establishing the anatomical pathways responsible for cortical modulation during STN and GP DBS lays a foundation for better understanding of therapeutic targets and thereby optimizing DBS electrode placement.

Disclosures: K. Kumaravelu: None. **C.S. Oza:** None. **C.E. Behrend:** None. **W.M. Grill:** None.

Poster

603. Mechanisms of ALS

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 603.01/V4

Topic: C.05. Neuromuscular Diseases

Support: ALS Association

National Institutes of Neurological Disease and Stroke

National Institutes of Health Common Fund

Title: Neurolincs imaging - illuminating human motor neuron biology

Authors: *S. FINKBEINER[1], .. NEUROLINCS CONSORTIUM[2], J. OSTERLOH[1];
[1]Gladstone Inst. of Neurolog. Dis., San Francisco, CA; [2]NIH, Bethesda, MD

Abstract: The goals for NeuroLINCS are to empirically determine key features that distinguish cellular states and to develop models for predicting how cells respond to perturbagens. We are interested in human iPSC-derived neurons from people with and without motor neuron disease. Using robotic microscopy (RM), we can identify and track individual cells over time and quantify the relationship between intermediate changes in cellular physiology and eventual fate. This quantitative molecular phenotyping enables us to construct multivariate predictive models of fate, which we will integrate with data generated from other NeuroLINCS sites, to provide comprehensive molecular "signatures" for our cell types of interest. Here, we present new image analysis techniques and cellular reporters that are illuminating new features of human motor neurons from patients with SMA and ALS, including those from sporadic patients and others with mutations in C9ORF72 and SOD1.

Disclosures: S. Finkbeiner: None. **.. NEUROLINCS Consortium:** None. **J. Osterloh:** None.

Poster

603. Mechanisms of ALS

Location: Halls B-H

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Topic: C.05. Neuromuscular Diseases

Support: NIH

ALS Finding a Cure

Robert Packard Center for ALS Research

ALSA

Travelers Insurance

National FootBall League

Professional Golf Association

Title: Answer ALS: Establishing a clinical and comprehensive multi-omics signature for ALS employing induced pluripotent stem cell derived motor neurons from 1000 sporadic and familial ALS patients nationwide

Authors: *J. D. ROTHSTEIN[1], M. CUDKOWICZ[3], C. SVENDSEN[4], N. MARAGAKIS[2], J. D. BERRY[3], L. THOMPSON[5], S. FINKBEINER[6], J. VAN EYKE[4], E. FRAENKEL[7], E. MOSMILLER[2], S. VAUGHN[3], T. THOMPSON[5], S. FARR[1], E. BAXI[1];
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Abstract: ALS, like many other neurodegenerative diseases, likely represents a collection of different subtypes of patient populations and molecular etiologies. Over a dozen different genetic mutations cause familial ALS (fALS) and fALS is clinically indistinguishable from the far more common sporadic ALS. Disappointingly, no new drug treatments have been found to be reproducibly successful in large clinical trials since the first and only FDA approved drug, more than 20 years ago. Using approaches gleaned from personalized medicine approaches in cancer, Answer ALS was conceived and organized as a comprehensive multi-omics approach to ALS to ascertain, at a population level, the various clinical-molecular- biochemical subtypes of ALS. The overall organization was built on the collaborative NIH initiated NeuroLinc's consortium. Specifically, ALS patients nationwide are being enrolled at 6 University clinics distributed throughout the USA and longitudinally followed with deep clinical data collection. In addition,

patients wear a personal health monitoring device with a linked Android/iOS app collecting 24/7 data on motor activity, sleep activity, heart rate, motor performance and learning "games", voice and pulmonary function. The iPS derived neurons are centrally generated from a novel, rapid and highly reproducible specific differentiation protocol. Whole genome sequencing, transcriptomics, epigenomics, proteomics, metabolomics, lipomics, high content imaging and longitudinal high throughput single cell analysis are collected on the patients iPS motor neurons also employing standardized and parallel cultures. Integrated clinical and biological signatures are being generated using bioinformatics, statistics and computational biology to establish patterns that may lead to a better understanding of the underlying mechanisms of disease. The data acquired in this consortium effort is open source and freely available online to academic and commercial researchers along with the library of patient derived iPS cells, all without IP restrictions. Ultimately the data will be analyzed using deep machine learning algorithms performed in collaboration with partner organizations. The overall goal of this comprehensive individualized clinical and biological national effort will be to identify biological subsets of ALS which will inform future clinical trials, help develop therapies targeting the proper molecular pathway for the right patient subgroup, provide a platform of human patient derived authentic neurons for use in patient subgroup drug discovery and appropriate biomarker and/or pharmacodynamic markers for use in clinical trials.

Disclosures: **J.D. Rothstein:** None. **M. Cudkowicz:** None. **C. Svendsen:** None. **N. Maragakis:** None. **J.D. Berry:** None. **L. Thompson:** None. **S. Finkbeiner:** None. **J. Van Eyke:** None. **E. Fraenkel:** None. **E. Mosmiller:** None. **S. Vaughn:** None. **T. Thompson:** None. **S. Farr:** None. **E. Baxi:** None.

Poster

603. Mechanisms of ALS

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 603.03/V6

Topic: C.05. Neuromuscular Diseases

Support: NIH Project 1U54NS091046-01

Title: Proteomic analysis of motor neurons from induced pluripotent stem cells: ALS and SMA

Authors: ***V. J. DARDOV**[1,2], V. VENKATRAMAN[2], R. HO[2], A. D. MATLOCK[2], .. NEUROLINCS CONSORTIUM[3], C. SVENDSEN[2], J. VAN EYK[2];
[1]Biomed. Sci., [2]Cedars Sinai, Los Angeles, CA; [3]NIH, Bethesda, MD

Abstract: Amyotrophic lateral sclerosis (ALS) is an adult onset neurodegenerative condition in which loss of upper and lower motor neurons occurs, while Spinal Muscular Atrophy (SMA) affects only the lower motor neurons and occurs in infants and young children. In both diseases, there is loss of neurons due to cell death which ultimately leads to impaired muscle function. Determining if and how these two diseases are linked at the molecular level can be investigated by employing cell wide proteomic analysis to determine the effects of single genetic perturbations (C9 and SMN1) that drive the ALS and SMA disease phenotype. Ideally the molecular profile of affected motor neurons in disease would be interrogated in vivo, but this is challenging and precludes experimentation to deduce cause and effect. iPSC derived motor neurons allow for the interrogation of the discrete molecular effects of specific genetic perturbations within the context of human genomic backgrounds upon which disease is made manifest, and furthermore enables chemical and environmental perturbations to further elucidate molecular pathology in ALS. The goal of this study was to identify disease specific molecular signatures characteristic of known disease phenotypes including neuronal cell death in an iPSC-derived motor neuron model of ALS and SMA. We have generated motor neuron precursors (iMNs) from the iPSCs derived from healthy human patients as well as those with SMA and ALS, (n=4/group). We hypothesize that by quantitative proteomic analysis we will elucidate the disease specific pathways and through bioinformatics analysis, be able to link the convergent and divergent pathways that lead to MN cell death in both ALS and SMA when compared to control. In this study, we used the cutting edge proteomic approach, Data Independent Acquisition Mass Spectrometry (DIA-MS, also referred to as SWATH), and by first constructing a iMN sample specific DIA peptide assay library we used a targeted analysis approach to quantitate 3934 proteins across the 12 iMN samples. Our quantified proteome included key neuronal markers, such as MAP2, DCX and Snap25 and enriched protein classes include vesicle coat proteins, SNARE proteins, and non-motor microtubule binding proteins. Future studies include fingerprinting the molecular pathology of the disease at earlier stages in culture by comparing this set of iMN data to its iPSC counterpart in an effort to tease out any pathways that are affected by cell maturation and development, as well as reveal disease specific pathways that can then be associated with single genetic perturbations.

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Poster

603. Mechanisms of ALS

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Program#/Poster#: 603.04/V7

Topic: C.05. Neuromuscular Diseases

Support: NIH

Answer ALS

Title: Integrative epigenomic and transcriptomic analysis to generate cell signatures from ALS iPS-derived motor neurons

Authors: *R. G. LIM[1], R. ESCALANTE-CHONG[2], J. WU[1], M. CASALE[1], P. MILANI[2], N. PATEL-MURRAY[2], A. M. REYES-ORTIZ[1], J. STOCKSDALE[1], T. THOMPSON[1], B. SHELLEY[3], L. ORNELAS[3], C. SVENDSEN[3], E. FRAENKEL[2], L. M. THOMPSON[1], .. NEUROLINCS CONSORTIUM[4];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disease impacting motor neurons in the brain and spinal cord, with ensuing paralysis and death. With the advancements in human induced pluripotent stem cell technologies, there is a unique opportunity to define the molecular networks that govern cellular responses to disease. Through the NeuroLINCS consortium, a series of molecular, cellular and data analytic approaches are being used to generate cell signatures from human brain cells derived from unaffected individuals and subjects with ALS or spinal bulbar muscular atrophy (SMA). We first used differentiated motor neurons from individuals with an ALS-causing hexanucleotide repeat expansion in *C9orf72* to investigate how this dominant mutation affects the epigenomic landscape that may drive transcriptomic dysregulation. iPSC-derived motor neurons from ALS and control patients were analyzed using total RNAseq to define changes in gene expression and alterations in exon usage and splicing. In parallel, ATAC-Seq, which reveals regions of open chromatin, was performed to identify epigenomic changes in the disease state versus control. In order to gain mechanistic insights into the relationship between chromatin accessibility and gene expression changes, we developed a tool called AChroMap (accessible chromatin mapper of transcriptional regulators), which allows one to determine relevant transcriptional regulators for a set of genes across disease states. We have applied AChroMap to identify potential important regulators in the context of the *C9orf72* expansion that may drive transcriptional changes and contribute to potential disease related phenotypes. These approaches will be expanded to integrate proteomic, lipidomic, and metabolomic data, as well as whole genome sequencing, single cell imaging, and functional cellular readouts with various perturbations, and all of these data will be made available to the scientific community through NeuroLINCS and Answer ALS to further elucidate the pathogenic mechanisms in ALS motor neurons.

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Poster

603. Mechanisms of ALS

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Topic: C.05. Neuromuscular Diseases

Support: NIH Grant U54NS091046-01

ALSA 11000 GE232

Title: Banking and distribution of motor neurons derived from induced pluripotent stem cells: A focus on motor neuron disease

Authors: *B. C. SHELLEY[1], B. MANDEFRO[1], L. SHUE[1], M. BANUELOS[1], D. WEST[1], L. ORNELAS[1], D. SAREEN[1], C. N. SVENDSEN[1], .. NEUROLINCS CONSORTIUM[2]; [1]Board of Governors Regenerative Med. Inst., Cedars-Sinai Med. Ctr., Los Angeles, CA; [2]NIH, Bethesda, MD

Abstract: There are many challenges involved in the production of valuable, consistent cell products that may be used to model diseases across different laboratories. We have addressed some of these challenges by creating a Production Core for human induced pluripotent stem cell (iPSC)-derived motor neurons that have been optimized for disease modeling studies and translational work. In particular, we are focusing our efforts on producing banks of motor neurons derived from patients with motor neuron diseases such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) using cutting-edge differentiation techniques for derivation and banking. Here, we describe our banking and quality control methodology with a focus on batch-to-batch consistency. Key aspects to the process include: standard operation procedure development and implementation, the initiation of a laboratory information management system (LIMS) for tracking manufacturing metadata throughout the growth process, and quality control measures such as short tandem repeat (STR) testing and immunocytochemistry for key motor neuron markers. Alongside these differentiation protocol optimizations, we are also developing genome-wide methods to assay global mRNA and protein expression in these cells. These analyses aim to sensitively gauge technical variability across batches, assess similarities to in vivo motor neurons, and detect biologically meaningful differences between disease and control conditions. Accounting for all these factors is critical for effective motor neuron-disease modeling.

Disclosures: **B.C. Shelley:** None. **B. Mandefro:** None. **L. Shue:** None. **M. Banuelos:** None. **D. West:** None. **L. Ornelas:** None. **D. Sareen:** None. **C.N. Svendsen:** None. **.. Neurolincs consortium:** None.

Poster

603. Mechanisms of ALS

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Topic: C.05. Neuromuscular Diseases

Support: AP Giannini Foundation

ALS Association

TargetALS

National Institutes of Neurological Disease and Stroke

NIH Common Fund

Title: Uncovering mechanisms of toxicity in C9ORF72 ALS.

Authors: *J. OSTERLOH[1], S. FINKBEINER, 94158[2];
[1]Inst. for Neurolog. Dis., [2]Gladstone Inst., San Francisco, CA

Abstract: Amyotrophic lateral sclerosis (ALS) is an incurable motor neuron (MN) disease that affects approximately 30,000 people in the USA. It manifests clinically as progressive muscle weakness and atrophy and results in fatal respiratory failure typically within 5 years of onset. Expansions of hexanucleotide repeats in the C9ORF72 gene are the leading cause of ALS, accounting for 8% of sporadic and 40% of familial ALS cases. However, the underlying mechanisms driving MN death due to C9ORF72 expansions are unknown. Three hypotheses regarding the mechanism of motor neuron death have emerged: (1) loss of normal C9ORF72 function; (2) sequestration of RNA-binding proteins by transcribed hexanucleotide repeats; and (3) expression of toxic dipeptide repeats translated from the hexanucleotide sequence. Here, we use Robotic Microscopy to assess the relative contributions of each potential mechanism to cell death, as both expression of transcribed but not translated GGGGCC repeats and translated non-GGGGCC dipeptide repeats are both highly toxic. Together, this powerful longitudinal, single-cell analysis provides novel insights into the specific cellular dysfunctions and dynamics that drive cell death due to C9ORF72 expansions.

Disclosures: J. Osterloh: None. **S. Finkbeiner:** None.

Poster

603. Mechanisms of ALS

Location: Halls B-H

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Program#/Poster#: 603.07/DP04 (Dynamic Poster)

Topic: C.05. Neuromuscular Diseases

Title: Maturation of iPSC-derived motor neurons co-cultured with brain microvascular endothelial cells in micro-engineered ALS-Chip.

Authors: *S. SANCES[1], G. VATINE[1], R. BARRILE[2], D. WEST[1], A. LAPERLE[1], R. HO[1], C. LUCCHESI[2], C. HINOJOSA[2], N. WEN[2], J. KERNS[2], G. A. HAMILTON[2], C. N. SVENDSEN[1]; [1]Cedars Sinai Med. Ctr., West Hollywood, CA; [2]Emulate, Inc., Boston, MA

Abstract: Traditional *in vitro* systems used in human stem cell-based modeling of neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS) possess inherent limitations for biological and pathological relevance. Studies have revealed that stem cell-derived neural tissue is unable to mature fully *in vitro*. This fetal-like immature phenotype presents a challenge when studying genetic contribution to adult-onset pathogenesis *in vitro.* Here, we hypothesize that iPSC-derived motor neurons (MNs) can better mature through enhanced endogenous media conditioning and the addition of developmentally relevant, non-neuronal cell types in co-culture. To address this hypothesis, we developed a micro-engineered co-culture system, termed "ALS-Chip". Calcium imaging and patch electrophysiology were used to characterize the functional effects of micro-media volumes on the neuronal maturation of induced pluripotent stem cell (iPSC)-derived MNs originating from non-disease control and ALS patients. We then developed a robust co-culture paradigm with iPSC-derived brain microvascular endothelial cells (BMECs) that forms a functional blood brain barrier across a porous membrane separating two distinct culture chambers. We interrogated the contribution of BMEC co-culture to MN maturation through morphology, transcriptomic analysis and electrophysiology. In addition, we introduced laminar flow in the ALS-Chip and studied the transcriptional effects of flow on both cell types and compared this to a traditional 96-well culture. The establishment of this novel ALS-Chip *in vitro* modeling strategy permits further studies into ALS disease mechanisms and blood brain barrier-related therapy development.

Disclosures: **S. Sances:** 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; Emulate. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Emulate. **G. Vatine:** None. **R. Barrile:** None. **D. West:** None. **A. Laperle:** None. **R. Ho:** None. **C. Lucchesi:** None. **C. Hinojosa:** None. **N. Wen:** None. **J. Kerns:** None. **G.A. Hamilton:** None. **C.N. Svendsen:** None.

Poster

603. Mechanisms of ALS

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Topic: C.05. Neuromuscular Diseases

Support: Live Like Lou Center for ALS Research

Target ALS Foundation

Frick Foundation for ALS Research

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Title: C9orf72 G_4C_2 HRE-mediated nucleocytoplasmic trafficking defects alter autophagic targeting

Authors: *J. R. MANN[1], A. M. GLEIXNER[1], M. R. MARKS[1], U. B. PANDEY[2], C. J. DONNELLY[1];
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Abstract: Working in conjunction with the ubiquitin-proteasome system (UPS), the autophagy-lysosomal pathway (ALP) plays a crucial role in the targeted degradation of damaged or aggregated proteins and dysfunctional organelles (1). Dysregulation of the ALP has been implicated in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS), a progressive and fatal neurodegenerative disease affecting motor neurons of the brain and spinal cord (2). The G4C2 hexanucleotide repeat expansion (HRE) in C9orf72 is the most common genetic cause of familial and sporadic forms of ALS, accounting for up to 30% of familial and 8% of sporadic cases (3). Toxicity associated with expression of this C9orf72 HRE has recently been linked to impairments in nucleocytoplasmic trafficking that result in the nuclear retention of RNA species and sluggish import of proteins destined for the nucleus (3-4). Interestingly, these nuclear import defects correlate with enhanced levels of cytoplasmic TDP-43, a pathological hallmark of roughly 97% of all ALS patients (5-6). While the exact mechanisms underlying these nuclear transport defects remains unclear, both G4C2 RNA and dipeptide repeat protein products of the repeat expansion have been linked to this dysfunction. Given the role of autophagy in the degradation of aggregated cytoplasmic proteins (7), it is possible that C9orf72-mediated mislocalization of aggregate-prone proteins such as TDP-43 may alter the degradation profiles of autophagosomes responsible for delivering cytoplasmic components to the lysosome for degradation. We developed a simple immunoprecipitation-based method to isolate pure and intact autophagosomes and found that these LC3-positive autophagosomal vesicles contain diverse protein and RNA species. Using this technique in conjunction with screening

methodologies, we assessed how G4C2 RNA, C4G2 RNA, and their repeat-associated non-ATG translation (RANT) protein products altered the autophagosome degradation profiles in cell culture and iPSC neurons. In addition, we tested whether expression of WT and ALS-linked mutant TDP-43 and FUS proteins revealed common autophagosomal targets. Alterations in degradation profiles are currently being confirmed in ALS-patient CNS tissue regions that show G4C2/C4G2 RNA foci or RANT protein accumulation. The data generated from this work will be used to catalog unique and common targets of the ALP among genetic subtypes of ALS.

Disclosures: J.R. Mann: None. **A.M. Gleixner:** None. **M.R. Marks:** None. **U.B. Pandey:** None. **C.J. Donnelly:** None.

Poster

603. Mechanisms of ALS

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 603.09/V11

Topic: C.05. Neuromuscular Diseases

Support: Live Live Lou Center for ALS Research

Target ALS Foundation

Frick Foundation for ALS Research

Genentech Medical Education and Research Grants

Title: Assessment of FG nup function in C9ORF72 ALS

Authors: *A. GLEIXNER[1], A. LUCE[1], J. R. MANN[1], M. R. MARKS[1], U. PANDEY[2], C. J. DONNELLY[1];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Pediatrics, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease characterized by the degeneration of the motor neurons and interneurons in the brain and spinal cord. The majority of ALS occurs sporadically (sALS; 90%) with no family history. Genetic studies of patients with a family history of ALS, familial ALS (fALS; 10%) have been studied to identify ALS-causing mutations within the human genome. Despite 32 known ALS-causing mutations, a G_4C_2 repeat expansion in the first intron of the *C9orf72* gene has been identified as the most common known genetic cause of both fALS and sALS comprising 30% and 8% of cases, respectively. A molecular mechanism conferring neurotoxicity for the repeat

expansion mutation is the generation of toxic G_4C_2 RNAs that sequester RNA binding proteins and the accumulation of dipeptide repeat proteins (DPRs) through the non-canonical repeat associated non-ATG translation (RANT) pathway. Recent studies show that the expression of G_4C_2 or arginine-rich DPRs, products of the *C9orf72* repeat expansion, dramatically alter the nucleocytoplasmic transport pathway. This results in the nuclear retention of RNAs and the reduction in the rate of nuclear import of proteins that contain a classical nuclear localization sequence (NLS) and the cytoplasmic enrichment of TDP-43. Moreover, components of the nuclear pore complex (NPC) were identified as potent modifiers of both nuclear transport defects and neurodegeneration in C9orf72 ALS *Drosophila* models. The NPC functions to regulate transport of molecules >40kDa across the nuclear membrane. It is comprised of thirty different nucleoporins and approximately half of the nucleoporins contain intrinsically disordered phenylalanine-glycine repeat domains (FG domains). FG Nups comprise the selective barrier of the NPC and their dysfunction alters the compartmentalization of nuclear and cytoplasmic proteins. Loss of some FG nucleoporins (FG nups) have been shown to modulate degeneration in C9ORF72 ALS *Drosophila* models. In this work, we begin to address the role of FG Nups in C9orf72 ALS pathobiology. Here, we employed a genetic screen in RNA and DPR *Drosophila* models of C9orf72 ALS and iPSC motor neurons to determine FG Nups important for C9orf72 mediated neurodegeneration. Next we assessed how C9orf72 RNA and/or DPR products alter FG Nup biology including stability and post-translational status. Finally, we determine if modulating FG Nup function and contributes to or rescues nucleocytoplasmic trafficking deficits in C9ORF72 ALS.

Disclosures: **A. Gleixner:** A. Employment/Salary (full or part-time): University of Pittsburgh School of Medicine, Live Like Lou Center for ALS Research. **A. Luce:** None. **J.R. Mann:** A. Employment/Salary (full or part-time): University of Pittsburgh School of Medicine, Live Like Lou Center for ALS Research. **M.R. Marks:** A. Employment/Salary (full or part-time): University of Pittsburgh School of Medicine, Live Like Lou Center for ALS Research. **U. Pandey:** A. Employment/Salary (full or part-time): University of Pittsburgh School of Medicine. **C.J. Donnelly:** A. Employment/Salary (full or part-time): University of Pittsburgh School of Medicine, Live Like Lou Center for ALS Research.

Poster

603. Mechanisms of ALS

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Program#/Poster#: 603.10/V12

Topic: C.05. Neuromuscular Diseases

Support: Live Like Lou Center for ALS Research

Target ALS Foundation

Frick Foundation for ALS Research

Genetech Medical Education and Research Grants

Title: Defining the pathways responsible for the nuclear export of g4c2 rna in c9orf72 als/ftd ipsc neurons

Authors: *M. R. MARKS[1,2], J. R. MANN[1,2], A. M. GLEIXNER[1,2], C. J. DONNELLY[1,2]; [1]Neurobio., Univ. of Pittsburgh, Pittsburgh, PA; [2]Live Like Lou Ctr. for ALS Res., Univ. of Pittsburgh Brain Inst., Pittsburgh, PA

Abstract: The G_4C_2 repeat expansion in the *C9ORF72* gene has been identified as the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), diseases that cause the degeneration of motor neurons and/or cortical neurons of affect patients. The C9ORF72 hexanucleotide repeat expansion is thought to act through a gain of function mechanism where the G_4C_2 RNA and/or its dipeptide repeat protein products (DPRs) impair the function of nuclear pore complexes (NPC). This leads to increased nuclear retention of cellular RNAs and a reduction in the nuclear import of proteins containing a classical nuclear localization sequence (NLS). Recent studies have revealed that genetic correction of nucleocytoplasmic transport via the enhancement of protein import is neuroprotective to G_4C_2-expressing *Drosophila.* Furthermore, studies in *Drosophila* expressing an intronic G_4C_2 sequence suggest nuclear retention of G_4C_2 RNA is neuroprotective. Taken together, these data indicate that the nuclear export of G_4C_2 RNA might be a key toxic mechanism that underlies C9ORF72 gain of function neurotoxicity. Here we aim to study the mechanism of G_4C_2 RNA nuclear export employing iPSC motor neurons from C9ORF72 ALS patients. The G_4C_2 RNA has been shown to form hairpins and G-quadruplex structures and might therefore highjack a number of nuclear export pathways for similar structured RNAs. To test this, we modulated critical components of the export machinery for mRNA, tRNA, snRNA, miRNA, and rRNA and assessed G_4C_2 RNA foci localization and DPR accumulation. In addition, we evaluated whether reducing the nuclear export of G_4C_2 RNA rescues nucleocytoplasmic trafficking defects previously observed in C9ORF72 iPSC neural cultures. Thus, if inhibiting G_4C_2 RNA export rescues these defect, it may act as a novel strategy for therapeutic development for C9ORF72 ALS/FTD.

Disclosures: **M.R. Marks:** None. **J.R. Mann:** None. **A.M. Gleixner:** None. **C.J. Donnelly:** None.

Poster

604. Neuroprotective Mechanisms: Models of Neurotoxicity, Excitotoxicity, and Stroke

Location: Halls B-H

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Program#/Poster#: 604.01/V13

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH IAA #AOD14018-001-00000

Title: Determining the minimum neuroprotective dose of n6-cyclopentoadenosine that prevents seizure after exposure to soman and vx nerve agents.

Authors: *T. P. THOMAS[1], T.-M. SHIH[2];
[1]Army Res. Lab., Aberdeen Proving Ground, MD; [2]US Army Med. Res. Inst. of Chem. Def., Aberdeen Proving Ground, MD

Abstract: Nerve agents, such as soman (GD) and VX, produce toxic effects via acetylcholinesterase inhibition. Current medical countermeasures display limited efficacy in preventing seizure and neuropathology. Previously, we showed that N6-cyclopentoadenosine (CPA), an agonist for the A1 adenosine receptor (AR), effectively prevented seizure and neuropathology at 55-60 mg/kg (intraperitoneally) in a GD seizure rat model. Although CPA was neuroprotective at that high dose, there were unwanted side-effects such as sedation and depression of cardiac output. This study aimed to determine if lower doses of CPA could prevent GD-induced seizure with fewer side-effects. Therefore, we conducted a dose escalation experiment to determine the minimum effective dose of CPA that prevented excitotoxicity after GD exposure for 24 hrs. Specifically, rats received CPA at 25, 30, 40, 50, or 60 mg/kg 1 minute after a 1.6xLD_{50} dose of GD. For control, rats received saline (i.e., 0 mg/kg CPA). Full protection from GD seizure was only achieved at 60 mg/kg (N=10). At 0, 30, 40, and 50 mg/kg, seizure rates were 100% (N=12), 67% (N=6), 50% (N=4), and 40% (N=5) respectively. Although CPA at 40 and 50 mg/kg did not prevent 100% seizure, all rats survived until the study's endpoint (at 24 hrs)-a significant improvement over the control group (0% survival). Whereas rats receiving 0 mg/kg CPA displayed severe cholinergic symptoms (e.g., salivation), hypersecretions were suppressed by CPA treatment at doses ≥ 40 mg/kg. Consequently, peripherally administered CPA may provide compressive protection by mitigating both peripheral and central cholinergic crises. Since the efficacy of neuroprotectants may vary between G and V nerve agents, we also evaluated CPA's efficacy against a challenge of VX at 1.6xLD_{50}. Investigation started by comparing the effects of CPA 0 mg/kg (control) to 60 mg/kg after VX. Twelve of the 14 controls experienced seizure and 7 survived 24 hrs. CPA at 60 mg/kg provided complete protection from seizure, and 10 of the 13 rats survived 24 hrs. Moreover, rats receiving 60 mg/kg treatment did not display peripheral signs. The success of 60 mg/kg of CPA prompted a dose reduction study where CPA was lowered until seizure activity was detected. In

all, we tested 60, 50, 40, 30, 20, 10, and 5 mg/kg CPA. Seizure was only detected in rats receiving CPA at 5 mg/kg. At that dose, 50% of the rats experienced intermittent seizure activity. The minimum effective dose that prevented all VX seizure was 10 mg/kg. The mechanism for CPA's marked efficacy at significantly lower doses for VX vs. GD requires further study. Although the doses differ, CPA proved to be a highly protective treatment strategy for both G and V agents.

Disclosures: T.P. Thomas: None. **T. Shih:** None.

Poster

604. Neuroprotective Mechanisms: Models of Neurotoxicity, Excitotoxicity, and Stroke

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 604.02/V14

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant NS076448

Title: Brief exposure to isoflurane as a post-exposure treatment against organophosphate chemical threat agents.

Authors: *J. KRISHNAN[1], J. R. MOFFETT[2], T. H. FIGUEIREDO[2], A. P. APPU[2], P. ARUN[2], M. F. BRAGA[2], A. M. NAMBOODIRI[2];
[1]APG, USUHS, Bethesda, MD; [2]APG, USUHS, Betheda, MD

Abstract: Organophosphate poisoning is a significant world health problem, claiming thousands of lives per year through intentional and unintentional pesticide exposure. Potential for a terrorism based release scenario has made the situation worse. Organophosphate based chemical threat agents (CTA) exert their toxic effects through cholinergic over-activation during the initial phase. However, if not treated immediately, shifts to a glutamatergic phase which can lead to status epilepticus (SE), irreversible neuronal degeneration and long term CNS damage. Prognosis for any CTA-based post exposure treatment is poor since most drugs fail to inhibit the post exposure phase of non-cholinergic activation. Recently, we have reported that intranasal delivery of obidoxime (OBD) provides almost 100% neuroprotection against lethal dose of paraoxon when administered 5 mins after intoxication. In subsequent studies, we have examined the post exposure time window of effectiveness and found that OBD was not effective at 30 min. During these studies, we have discovered that isoflurane, the anesthetic used for the intranasal delivery, was very effective as a post exposure neuroprotectant. Sprague Dawley rats (250 ±40g) were exposed to lethal dose of paraoxon (4mg/kg) and were randomly assigned to control group and isoflurane treated group with a time course from 10min to 2 hours. Isoflurane treatment involved

2% isoflurane exposure for 3 mins followed by 5% for 1 min in 100% oxygen. All animals exhibited at-least stage 3 seizures, according to modified Racine scale until they were treated with isoflurane. Post exposure Isoflurane treatment decreased seizure severity significantly with the peak effectiveness when isoflurane was given around 30 min post exposure. Animals were euthanized at 24 h following transcardial perfusion using 4% formaldehyde and Fluoro-Jade-C staining was performed in brain slices. There were extensive neuronal degeneration in the surviving untreated control animals 24 hours after paraoxon administration, whereas degenerating neurons decreased significantly in the isoflurane treated groups, with the greatest reduction at around 30 min post treatment period. Dose -response studies indicated that exposure to 2% isoflurane for 3 min followed by 1 min exposure in the -3.5 to 5% range is effective. Studies are in progress to determine the possible mechanisms involved. Our subsequent goal is to develop isoflurane as a post exposure treatment for CNS damage caused by CTA exposure in view of the lack of toxicity due to the brief exposure required, noninvasive nature and attractiveness for field applications in a terrorism scenario.

Disclosures: J. Krishnan: None. **J.R. Moffett:** None. **T.H. Figueiredo:** None. **A.P. Appu:** None. **P. Arun:** None. **M.F. Braga:** None. **A.M. Namboodiri:** None.

Poster

604. Neuroprotective Mechanisms: Models of Neurotoxicity, Excitotoxicity, and Stroke

Location: Halls B-H

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Program#/Poster#: 604.03/V15

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: U54NS083924

5 G12 RR 003035-28

8G12 MD 007583-28

Title: A brain slice model of gulf war illness allows for mechanistic studies and the search for antidotes

Authors: *P. A. FERCHMIN[1], D. PEREZ[1], M. CARRASCO[1], M. PEREZ LASSALLE[1], H. A. MARTINS[2], V. A. ETEROVIC[1];
[1]Biochem., Univ. Central Del Caribe, Bayamon, PR; [2]Pharmacol. & Toxicology, Univ. of Puerto Rico - Med. Sci. Campus, San Juan, PR

Abstract: Gulf War Illness (GWI) is a neurological syndrome afflicting about 25% of veterans from the Gulf War (1990-1991). The etiology of the GWI are the neurotoxicants to which the

veterans were exposed. The relevant neurotoxicants include traces of sarin, pyridostigmine (PB), N,N-diethyl-meta-toluamide (DEET), and permethrin (Per). Here diisopropylfluorophosphate (DFP), a surrogate of sarin, was included instead of sarin. The objective of the present project was to study the mechanism of GWI with the aim of developing antidotes to alleviate the GWI. The method used was an ex-vivo model which is faster and less costly than classical in vivo models. The neurotoxicity and neuroprotection were assessed by recording population spikes (PS) from acute hippocampal slices. PSs are the sum of the synaptically elicited axon potentials of a population of neurons. Axon potentials are all-or-none robust responses produced by functional neurons. The loss of PSs is an early event that precedes neuronal death which can be prevented by neuroprotective compounds. Remarkably, the neuroprotective and neurotoxic effects observed in slices have repeatedly been confirmed in vivo confirming the usefulness of this method. All the chemicals implicated in the GWI showed toxicity in this hippocampal slice model. The order of toxicity was DFP>PB>Per>DEET. The GWI drugs were tested by application to the slices for 2 hours in μM concentrations followed by 1 hour of the presumed antidotes. GWI drugs decreased the PS in a concentration-dependent manner. However the application of edelfosine, flupirtine or (1S,2E,4R,6R,7E,11E)-cembra-2,7,11-triene-4,6-diol (4R) restored 80-90% of the initial PSs. Edelfosine is an inhibitor of PLC-beta3, flupirtine is an activator the Kv5.2-7 channels and 4R is a neuroprotective, and anti-inflammatory cembranoid developed by our group. Our data suggest that the pivotal component of the GWI neurotoxicity is the over-activation of PLC-beta3 and inhibition of Kv5.2-7 channels. In slices, this causes glutamatergic excitotoxicity and inflammation. A chronic in vivo exposure to GWI drugs will likely cause a pathological lingering glutamatergic excitotoxicity, and inflammation later expressed as a neurological deficit. Paradoxically the symptoms of the GWI continue in the absence of the neurotoxic agents indicating that the GWI is perpetuated by a vicious cycle. We suggest that the GWI symptoms could be ameliorated by reactivation of the Kv5.2-7 channels and decreasing inflammation.

Disclosures: **P.A. Ferchmin:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US 8,530,525 B2. Methods and composition for protecting and treating neuroinjury. **D. Perez:** None. **M. Carrasco:** None. **M. Perez Lassalle:** None. **H.A. Martins:** None. **V.A. Eterovic:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder.

Poster

604. Neuroprotective Mechanisms: Models of Neurotoxicity, Excitotoxicity, and Stroke

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 604.04/V16

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NICHD 1R41HD079201

Title: Human chorionic gonadotropin protects the neonatal brain against hypoxic-ischemic neurodegeneration and inhibits glutamate-dependent excitotoxic neuronal cell death

Authors: *R. GALINDO[1], T. Z. MOVSAS[2], R. WEINER[3], D. M. HOLTZMAN[3]; [1]Neurol., Washington Univ. In St. Louis, Saint Louis, MO; [2]Zietchick Res. institute, LLC, Plymouth, MI; [3]Washington Univ., St. Louis, MO

Abstract: Neonatal cerebral hypoxia-ischemia (H-I) is a major cause of perinatal mortality and neurodevelopmental dissability in children although limited effective strategies are available for its prevention and treatment. The placental hormone human chorionic gonadotropin (hCG) is a glycoprotein with potential neuroprotective properties with recent data demonstrating beneficial effects in animal models of adult stroke. In the present study we ask whether hCG acts as a neuroprotectant against the effects of neonatal cerebral H-I. We tested this hypothesis in two ways. First, we used the modified Levine model of newborn H-I to examine the protective effects of in *vivo* hCG in the term-equivalent neonatal mouse. Second, we examined the direct actions of hCG against the neurodegenerative effects of glutamate-dependent excitotoxic neuronal injury *in vitro* utilizing the dissociated hippocampal and cortical neuron preparation. *In vivo* intraperitoneal (IP) injection of 1.5 IU/mg of hCG 18 hours prior to H-I decreased hippocampal and striatal tissue loss by 36% and 29% respectively. In addition, IP hCG administration increased cerebral levels of vascular endothelial growth factor mRNA, a molecule with known neuroprotective actions in the immature brain. The neuroprotective effect of hCG is possibly mediated via a glutamate-dependent mechanism given that *in vitro* administration of hCG at a physiological concentration (2 IU/mL) promoted the survival of immature cortical and hippocampal neurons following glutamate-dependent excitotoxicity. High doses of hCG did not adversely affect neuronal survival, but rather resulted in an increase in neuronal fiber sprouting. The above observations suggest that hCG may be a potential novel neuroprotective agent against the neurodegenerative effects caused by neonatal hypoxic-ischemic brain injury.

Disclosures: R. Galindo: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent: Use of human chorionic gonadotropin to treat cerebral palsy and/or its co-morbidities WO 2014116786 A1. **T.Z. Movsas:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent: Use of human chorionic gonadotropin to treat cerebral palsy and/or its co-morbidities WO 2014116786 A1. **R. Weiner:** None. **D.M. Holtzman:** None.

Poster

604. Neuroprotective Mechanisms: Models of Neurotoxicity, Excitotoxicity, and Stroke

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 604.05/V17

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: $GABA_A$ alpha4beta3delta receptors are a target to supplement standard medical countermeasures for status epilepticus caused by acute soman poisoning

Authors: H. MCCARREN[1], R. YOSHIMURA[2], D. HOGENKAMP[2], C. SMITH[1], *K. W. GEE[2], J. MCDONOUGH[1], T. JOHNSTONE[2,1];
[1]Res. Div., U.S. Army Med. Res. Inst. of Chem. Def., Aberdeen Proving Ground, MD;
[2]Pharmacol., Univ. of California Irvine, Irvine, CA

Abstract: The neurological outcome of acute organophosphorus (OP) poisoning is a state of chronic uncontrolled epilepsy in the brain (i.e., status epilepticus, SE). Standard medical countermeasures for OP-induced seizures include midazolam, a benzodiazepine (BZ) anticonvulsant with rapid onset of action and reasonable efficacy on the survival rate of victims. However, improvement of seizure control is needed at longer time points after nerve agent exposure to minimize the resulting lethal neuropathology. Soman (GD) exposure is reported to reduce surface expression of synaptic $GABA_A$ receptors ($GABA_AR$) in the hippocampus to impair $GABA_AR$ function. Therefore sufficient receptors responsive to BZs may not be available for receptor modulation after poisoning. We tested whether enhanced function at extrasynaptic delta-subunit containing $GABA_ARs$ could augment the control of nerve agent poisoning. Two high efficacy/high potency modulators of alpha4beta3delta $GABA_ARs$ were tested against pilocarpine-induced seizures in mice and acute GD poisoning in rats. UCI50027 (non-selective neurosteroid) or 2-261 (beta-subunit subtype selective enaminone) were co-injected with 1 mg/kg scopolamine 30 minutes prior to a 416 mg/kg dose of pilocarpine. Mice were placed in individual housing and tremors, clonic seizures, SE, tonic seizures and death were noted. In the GD studies, rats were prepared for brain electroencephalographic (EEG) waveform measurements then placed in chambers to record ~30 min of normal EEG baseline. The animals were then pretreated with the oxime HI-6 (125 mg/kg, IP) and 30 min later challenged with GD (180 ug/kg, SC) at a dose that elicits electrographic seizure activity in 100% of the animals. Twenty minutes following the onset of seizure activity the animals received standard medical countermeasures (0.45 mg/kg atropine sulfate admixed with 25 mg/kg, 2-PAM, IM; 1.8 mg/kg, midazolam, IM) for nerve agent intoxication with or without a test dose of UCI50027 or 2-261. Evaluation and categorization of the EEG response to treatment was performed (4 hrs) and rated by absence of all spiking and/or rhythmic waves. Inclusion of UCI50027 in the standard medical countermeasure terminated seizure activity in test animals and afforded protection from seizures supported by reductions in gamma power and decreases in mean spike frequency.

Alpha4beta3delta $GABA_A$Rs targeted compounds may augment standard medical countermeasures for GD poisoning by enhancing synaptic GABA efficacy of BZs and provide additional efficacy at extrasynaptic $GABA_A$Rs.

Disclosures: H. McCarren: None. **R. Yoshimura:** None. **D. Hogenkamp:** None. **C. Smith:** None. **K.W. Gee:** None. **J. McDonough:** None. **T. Johnstone:** None.

Poster

604. Neuroprotective Mechanisms: Models of Neurotoxicity, Excitotoxicity, and Stroke

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 604.06/V18

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Department of Defense W81XWH-12-1-0536

Title: Axonal transport, mitochondrial dynamics, and gulf war illness (*In vitro* studies)

Authors: *S. X. NAUGHTON[1], J. GAO[2], C. M. HERNANDEZ[1], A. V. TERRY, Jr[1];
[1]Pharmacol. and Toxicology, [2]Augusta Univ., Augusta, GA

Abstract: The disorder known as Gulf War Illness (GWI) affects about 25-30% of veterans deployed to the Gulf War in 1990-91 and is characterized by a variety of chronic symptoms including neurologic deficits. While a host of potential contributing factors have been implicated, the underlying pathophysiology of the specific symptoms of GWI has not been clearly established, thus limiting the development of effective treatment strategies. One compelling explanation for the neurological-based symptoms is exposure to acetylcholinesterase inhibitors (AChEIs) (Golomb et al., 2008) including organophosphates (OPs). Previous studies in our laboratory have shown that OPs (e.g., chlorpyrifos) can impair the transport of mitochondria in axons as well as lead to alterations in mitochondrial dynamics, suggesting potential mechanisms for the aforementioned neurologic deficits. Here, using a time-lapse imaging technique in cortical neurons, we evaluated chlorpyrifos (CPF) and its oxon metabolite (CPO) across a wide range of concentrations (subnanomolar to micromolar) for effects on fast axonal transport of membrane bound organelles (MBOs) that contained the amyloid precursor protein (APP) tagged with the fluorescent marker, Dendra2 (APPDendra2). 24 hours of exposure to CPF and CPO resulted in a decrease in the velocity of anterograde and retrograde movements of MBOs and an increase in the number of stationary MBOs. These effects of CPO occurred at picomolar (100 pM) to low nanomolar (0.1 nM) concentrations that were not associated with compromised cell viability or cytoskeletal damage. Moreover, the effects of CPO and CPF on axonal transport occurred at concentrations that did not inhibit AChE activity and they were not blocked by

cholinergic receptor antagonists. Given the fundamental importance of axonal transport to neuronal function, these observations may explain some of the long term neurological deficits that have been observed in GWI and in others exposed to organophosphates (e.g., farmers, pesticide applicators). In ongoing studies we are also seeking to elucidate the relationship between axonal transport and mitochondrial dynamics, and to utilize phenotypic screening measures to pursue therapeutic compounds which may reverse OP associated deficits within these domains.

Disclosures: **S.X. Naughton:** None. **J. Gao:** None. **C.M. Hernandez:** None. **A.V. Terry:** None.

Poster

604. Neuroprotective Mechanisms: Models of Neurotoxicity, Excitotoxicity, and Stroke

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Program#/Poster#: 604.07/W1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: USFDA/NCTR E07512.01

Title: Vascular endothelial degeneration in the rat striatum is associated with prolonged exposure to rotenone

Authors: *Z. K. BINIENDA, B. GOUGH, S. SARKAR;
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Abstract: The plant-derived pesticide, rotenone, is a potent mitochondrial toxin that leads to degeneration in striatal nerve terminals and nigral neurons. Rotenone-induced behavioral, neurochemical and neuropathological changes in rats mimic those observed in Parkinson's disease. We previously reported rotenone-induced decreases in tyrosine hydroxylase (TH) in the striatum as well as in the substantia nigra and a time-dependent depletion of striatal dopamine, concomitant with peripheral motor nerve dysfunction following a prolonged exposure. Rats were treated with rotenone at 1 mg/kg i.p. for 37 days. Control rats received vehicle (30% Solutol HS 15 in 0. 9% saline). A loss of dopamine transporter (DAT) and TH in the midbrain was observed along with activation of both microglia and astroglia after rotenone treatment. Here, striatal vascular integrity was evaluated in the same animals using a rat endothelial cell antigen -1 RECA-1 immunostaining method. Rats were perfused after treatment with 4% paraformaldehyde for immunohistochemical analyses. In the rotenone treated animals, RECA-1 immunolabeled endothelial cells were shrunken, regressed and thinner compared to those from vehicle controls. Image analysis using Image J software revealed that the % area of the RECA-1 positive vessels

was 4.53 ± 0.96 in rotenone treated vs. 9.05 ± 0.96 in control rats ($p<0.05$) and the average area was 22.21 ± 1.62 μm in treated vs. 33.51 ± 3.10 μm in controls ($p<0.05$). This study indicates that prolonged exposure to the neurotoxin, rotenone, not only decreases TH and DAT in the striatum but also affects endothelial cells in that area. The sequence of events, i.e., whether endothelial cell degeneration is caused by the neurodegeneration or the neurodegeneration and neuro-inflammation are concomitant with vascular damage has yet to be determined.

Disclosures: Z.K. Binienda: None. **B. Gough:** None. **S. Sarkar:** None.

Poster

604. Neuroprotective Mechanisms: Models of Neurotoxicity, Excitotoxicity, and Stroke

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Program#/Poster#: 604.08/W2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: The National Natural Science Foundation of China No. 81171090

The National Natural Science Foundation of China 81271460

Title: Foxo1-mediated inflammatory response after cerebral hemorrhage in rats

Authors: *J. ZHAO, Z. Y. LI, Q. HE, X. ZHAI, Y. YOU, Y. ZHAO, Y. H. HOU; CHONGQING MEDICAL UNIVERSITY, Shanghai City, China

Abstract: The forkhead box O (Foxo) family of transcription factors plays a crucial role in cell apoptosis, immune regulation, and tissue development. Foxo1, as the foremost member of the Foxo family, regulates a wide range of molecular signals in many tissues, including tumor, liver, and brain. This study investigated Foxo1 expression at different time points and in different brain areas, and the role of Foxo1 *in vivo* in regulating inflammatory injury in a rat model of autologous blood-injected cerebral hemorrhage injury. We found that Foxo1 expression peaked at 12 h post-intracerebral hemorrhage (ICH) and in the ipsilateral corpus striatum. Foxo1 knockdown by Foxo1 siRNA decreased ICH injury, improved neurological function, and decreased the expression of inflammatory factors downstream of the Foxo1 pathway, including TLR4, NF-κB, TNF-α, IL-1β, and IL-18. Foxo1 knockdown also decreased the expression and activity of myeloperoxidase, IL-1β, and IL-18. In conclusion, our findings demonstrate that Foxo1 is a key regulator of inflammatory injury in rats after ICH. By identifying the molecular mechanisms of Foxo1/TLR4/NF-κB signaling, we provide a novel rationale for therapeutic approaches to managing inflammatory injury after ICH.

Disclosures: **J. Zhao:** None. **Z.Y. Li:** None. **Q. He:** None. **X. Zhai:** None. **Y. You:** None. **Y. Zhao:** None. **Y.H. Hou:** None.

Poster

604. Neuroprotective Mechanisms: Models of Neurotoxicity, Excitotoxicity, and Stroke

Location: Halls B-H

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Program#/Poster#: 604.09/W3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NINDS 1R01NS071956-01

Title: Nanocoffee increases stem cell proliferation and displays neurogenic and neuroprotective effects in stroke models

Authors: *V. DE ALVARENGA GUEDES[1], J.-Y. LEE[1], M. PROVENZANO[3], I. ANTONUCCI[3], L. STUPPIA[3], K. RICHARDS[1], N. TAJIRI[1], C. CAO[2], C. V. BORLONGAN[1]; [1]Dept. of Neurosurg. and Brain Repair, [2]Dept. of pharmaceutical Sci., Univ. of South Florida, Tampa, FL; [3]Dept. of Psychological, Hlth. and Territorial Sci., G. d'Annunzio Univ., Chieti-Pescara, Italy

Abstract: Stroke is a leading cause of death and disability in the world. Despite the clinical relevance of stroke, available treatment options are limited, thereby making the development of novel therapies an urgent need. Epidemiological and animal studies have attributed neuroprotective properties to coffee. In the present study, we used well-established in vitro and in vivo ischemic stroke models to investigate the therapeutic potential of nanosized coffee (nanocoffee). In vitro, we exposed human amniotic-fluid stem cells (AFSCs) or rat primary cortical neurons to oxygen-glucose deprivation (OGD). AFSCs and cortical neurons were treated with different concentrations of nanocoffee and subsequently exposed to the OGD procedure for 90 minutes. Additionally, AFSCs were treated with nanocoffee for 24 hours in the absence of insult (normoxic condition, regular cell culture medium). Cell viability was measured using a calcein AM (Acetoxymethyl) assay. In vivo, adult male Sprague-Dawley rats were submitted to the middle cerebral artery occlusion (MCAo) model, which induces transient unilateral focal ischemia. Rats received a single intrajugular vein injection of nanocoffee or vehicle immediately after the beginning of the artery occlusion. All animals were euthanized 3 days after the MCAo for cresyl-violet staining and immunohistochemistry experiments using ki67 (cell proliferation marker) and doublecortin (neurogenesis marker). We observed that AFSCs ($p<0.01$) and cortical neurons ($p<0.05$) were protected against OGD-induced cell death by the nanocoffee treatment. Additionally, nanocoffee increased AFSCs proliferation under regular cell culture conditions ($p<0.01$). In vivo, we found an increase in both cell proliferation and neurogenesis in the

subventricular zone (SVZ), a neurogenic niche in the mammalian brain, with the nanocoffee treatment (p's<0.05). No significant effects of the nanocoffee treatment were observed on the number of live cells in the peri-infarct area as revealed by the cresyl violet staining. The present study shows that nanocoffee stands as a potent stem cell proliferative agent, displays proliferative and neurogenic effects in the SVZ, and acts as a neuroprotectant in an in vitro stroke model. Our results suggest that nanocoffee has a therapeutic potential in stroke. Studies of nanocoffee mechanisms of action are underway.

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Poster

604. Neuroprotective Mechanisms: Models of Neurotoxicity, Excitotoxicity, and Stroke

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Title: Bio activates the canonical wnt pathway to ameliorate neuronal cell death *In vitro* and following intracerebral hemorrhage

Authors: ***J. Y. ZHANG**[1], S. WON[1], Z. Z. WEI[1], Y. ZHOU[2], S. P. YU[1], L. WEI[1,2];
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Abstract: Hemorrhagic stroke, or intracerebral hemorrhage (ICH), is a devastating medical emergency that accounts for 15% of all stroke cases, but results in 40% of stroke-related mortality. Currently, no effective clinical treatments exist to counteract ICH. In the present investigation, we evaluated 6-bromoindirubin-3'-oxime (BIO) after ICH as a therapeutic candidate. BIO is a selective inhibitor of glycogen synthase kinase 3β (GSK-3β), a key intracellular signaling mediator of multiple pathways. One of the signaling pathways inhibited by GSK-3β is the canonical Wnt/β-catenin system, which is involved in transcriptional regulation of mitogenic and trophic genes, such as c-Myc and brain-derived neurotrophic factor (BDNF). Because BDNF promotes neuronal viability, we hypothesized that activation of the canonical Wnt pathway would confer neuroprotective effects. Specifically, we evaluated whether post-ICH

treatment with BIO could enhance cellular survival. The neuroprotective effects of BIO was firstly examined in primary cortical neuronal cultures subjected to oxygen-glucose deprivation (OGD). Cell death was measured at 24 hrs after OGD using the lactate dehydrogenase (LDH) assay and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay. BIO (0.1 - 10 μM, co-applied with OGD) significantly enhanced neuronal viability in a dose-dependent pattern, with the greatest neuroprotection observed at 10 μM. In addition, in primary astrocyte cultures subjected to OGD, activation of the canonical Wnt pathway did not show significant effect on astrocyte death, suggesting that the Wnt pathway is specific for neuronal viability. Next, we implemented BIO administration in an ICH model of adult mice, induced by injections of collagenase IV into the striatum. BIO (150 μL, 4 μM, i.p.) was co-administered during collagenase IV injection, and then the animals were sacrificed at 48 hr post-ICH for immunohistochemistry. In immunohistochemical staining of terminal deoxynucleotidyl transferase dUTP nick end labeling (*TUNEL*), cell death was examined by colabeling with the neuronal marker NeuN and astrocyte marker GFAP. The cytotoxicity was almost exclusively localized to neurons, with astrocytes undergoing minimal cell death. Importantly, BIO successfully ameliorated the magnitude of neuronal cell death and decreased the hemorrhage size and the area of necrotic tissue. Overall, this report provides new evidence for a translational potential of BIO following intractable brain injuries, such as ICH.

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Poster

604. Neuroprotective Mechanisms: Models of Neurotoxicity, Excitotoxicity, and Stroke

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR

Title: Inhibition of RGMa cleavage promotes axonal regeneration and improved neurological deficits after CNS injuries

Authors: ***N. G. TASSEW**[1], A. SHABANZADEH[2], P. BANERJEE[2], P. MONNIER[2];
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Abstract: RGMa inhibits outgrowth of many types of CNS neurons through interaction with its receptor, Neogenin. The RGMa/Neogenin pathway is involved in several processes both in the developing and mature CNS, including axon guidance, cell differentiation, cell survival, and

axonal regeneration. RGMa is processed into several fragments by a combination of autocatalytic and proprotein convertase (Furin and SKI-1) enzymatic cleavages. The ensuing membrane bound and soluble fragments interact with Neogenin and inhibit axonal outgrowth. Since RGMa is upregulated at the lesion site after CNS injury, we hypothesized that treatment with SKI-I inhibitor will prevent its activation following CNS injuries. The present study examined the therapeutic effects of SKI-1 inhibitor (PF 429242) in optic nerve crush and middle cerebral artery occlusion (MCAO) models. We show that inhibition of RGMa cleavage leads to axonal regeneration in optic crush model, and reduced infarct volume and improved neurological deficits following cerebral ischemia.

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Poster

604. Neuroprotective Mechanisms: Models of Neurotoxicity, Excitotoxicity, and Stroke

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: VA Merit review grant

Title: Effect of GW4869 in serum free DITNC1 astrocyte culture

Authors: *P. R. GUDA[1], S. RAY[2], O. A. KHAN[2], D. TRISLER[2,3,5], C. T. BEVER, Jr[2,4,5], T. K. MAKAR[2,4,5];
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Abstract: Astrocytes play a major role in the central nervous system (CNS). Astrocytes secrete pro-inflammatory cytokines in different neurodegenerative diseases. Astrocytes grown in serum-deprived (SD) media can cause apoptosis by elevating different apoptotic proteins. Furthermore, astrocytes secrete exosomes, membrane-enveloped Nano vesicles. Exosomes communicate cell to cell by transporting genetic material, proteins and lipids and subsequently regulating functions of targeted cells .Exosome release is decreased by the inhibition of sphingomyelinase2, an important enzyme responsible for formation of ceramide from sphingomyeline, by using GW4869. In DITNC1 cells, it is found that GW4869 treatment (72 hr,40uM) effectively prevented apoptotic cell death under SD conditions. Our objective is to determine the neuroprotection role of GW4869 on SD DITNC1 astrocyte cell line where cell death occurs in an

apoptotic manner by inhibiting exosome release. 40 μM GW4869 was used in serum free media of DITNC1 cell culture. The cells were incubated for 72 hours in SD with and without GW4869 treatment. Interestingly, we found the level of endothelin1 (ET1) was significantly increased in serum free media and that cytokine levels were decreased after GW4869 treatment. We also found that Bax, cleaved-caspase 3, cleaved-caspase 9, apoptotic markers and ET1, an inflammatory molecule, were increased in SD cells and that all of these parameters were reduced after GW4869 treatment. Furthermore, we observed that the Drp1, mitochondrial fission marker, expression was significantly increased in SD cells and was suppressed by GW4869 treatment. In the same experimental condition, in the cell medium exosome content was downregulated in SD cells by GW4869 treatment compared to without drug treatment. Exosomes were isolated from the cell supernatant by centrifugation at 100,000 g for 2 hour. Exosome markers GAPDH and CD63 were determined by western blot analysis. Our results suggest that GW4869 increases cell survival by preventing exosome release and indicate nSMase2 as a potential drug target for astrocyte associated neuroinflammatory diseases. Collectively, our findings suggest That GW4869 can be used for anti-inflammation and neuroprotection.

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Poster

604. Neuroprotective Mechanisms: Models of Neurotoxicity, Excitotoxicity, and Stroke

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Fondecyt 11400108

Title: NF-kB S-nitrosylation in excitotoxicity

Authors: *A. CAVIEDES, K. CORVALAN, B. MATURANA, U. WYNEKEN;
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Abstract: Introduction: Excitotoxicity, i.e. neuronal death by excessive NMDA receptor activation, neuronal Ca^{2+} overload and nitric oxide (NO) synthesis, is a prominent mechanism leading to cell death (Sandoval et al. 2011, J Neurochem 118:760). NO leads to proteins S-nitrosylation. We postulate that in hippocampal (i.e. vulnerable neurons) and cortical neurons (i.e. more resistant neurons), a differential set of proteins involved in cell death regulation is S-nitrosylated following an excitotoxic challenge. **Materials and methods:** To pull down hippocampal S-nitrosylated proteins, the biotin switch method was applied. Proteins were

identified by mass spectrometry and validated by Western blots. Viability test were performed with the Trypan blue exclusion test. **Results:** From a total of 863 proteins, 744 were detected only in one of the culture conditions. S-nitrosylation levels in hippocampal and cortical cultures were compared by Western blots: S-nitrosylation of the scaffolding protein PSD-95 and of the NMDA receptor subunit GluN2A increased after NMDA incubation in cortical and hippocampal cells. S-nitrosylation of the scaffolding protein SAPAP4 increased only in cortical cells, while S-nitrosylation of the NF-κB subunit p65 increased in cortical cells (i.e. its transcriptional activity was potentially inhibited) while it decreased in hippocampal cells. Moreover, the NF-κB inhibitor 6-(Phenylsulfinyl)tetrazolo[1,5-b]pyridazine prevented hippocampal neuronal death while it had no effect on viability of cortical neurons. **Conclusion:** The results reveal a notable specificity of NO targets that is cell-type dependent. Bi-directional regulation of protein S-nitrosylation and especially, of NF-κB pathway components, may constitute a major mechanism able to control excitotoxicity-related cell death. Fondecyt 11400108

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Poster

604. Neuroprotective Mechanisms: Models of Neurotoxicity, Excitotoxicity, and Stroke

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: EPO neuroprotection and MAPK signalig pathway in excitotoxic brain injury model mediated by MSG

Authors: *S. F. CORNELIO-MARTINEZ[1,2], M. RIVERA CERVANTES[2], C. BEAS ZARATE[2];
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[2]Biologia Celular y Mol., Univ. de Guadalajara, Guadalajara, Mexico

Abstract: Glutamate (Glu) is the main excitatory neurotransmitter in Central Nervous System (SNC). It has been show that play important role in many brain processes including differentiation, migration, survival, learning, plasticity memory and behavior. It function through two main types of Glu receptors (GluR), ionotropic and metabotropic. Excessive activation of GluR may excite nerve cells to their dead in a process referred as excitotoxicity. Thus Glu has also been implicated in neurodegenerative disorders like cerebral ischemia, traumatic brain injury, Huntington's disease, Alzheimer disease and epilepsy. These receptors triggering not only a significant Ca2+ influx but also the activity of transduction-signal cascade where the activation

of MAPK may promote S-phase entry of neurons, which leads to cell death. There are several in vitro and in vivo studies reporting neuroprotective effect of erythropoietin (EPO), through the activation of anti-apoptotic, anti-oxidant, and anti-inflammatory pathways as well as through the stimulation of angiogenic and neurogenic events. The main purpose of this work was to evaluate the MAPK signal cascade after the administration of excessive MSG, and determine the neuroprotective effect of EPO after excitotoxicity mediated through MSG. We evaluate the activation of MAPK cascade and the neuroprotective effect of EPO in the hippocampus of neonate male Wistar rats. First we evaluate the activation of MAPK signal cascade at 5, 6, 7 and 8 postnatal days (PD) after the administration of MSG at 1, 3, 5 and 7 PD using a selected PCR arrays, including MAPK signal pathway (PARN-061A) Rat Microarray MAP Kinase Signaling Pathway. Then we administered three different doses of EPO (250UI/kg body weight (bw), 500UI/kg bw and 1,000UI/kg bw) intravenously at 8 PD and determine the cell viability at 14 PD using Nissl technique, because it has been demonstrated that some regions of the hippocampus were more susceptible to cell damage at this age. Results of this work demonstrated that after the administration of MSG the MAPK signal cascade is activated and some of the molecular effectors of this cascade like Cdkn2, C-Fos, Ccnd2, Creb, Mapk13, Mapk10, Rac1 and Col1a1were up or down regulated. These molecules are related with cell cycle and may promote S-phase entry of neurons. We also demonstrated that after administration of EPO, cell damage in hippocampal CA1 region decrease compare with those animals that were administered with MSG only and the most effective dose of EPO was 1,000UI/kg bw.

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Poster

604. Neuroprotective Mechanisms: Models of Neurotoxicity, Excitotoxicity, and Stroke

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Adelson Foundation

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Title: Cell death after experimental hemorrhagic stroke *In vitro* is ferroptotic and necroptotic

Authors: *M. ZILLE[1], S. S. KARUPPAGOUNDER[1], Y. CHEN[1], T. A. MILNER[2], E. A. JONAS[3], R. R. RATAN[1];
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Brain and Mind Res. Inst., Weill Cornell Med. of Cornell Univ., New York, NY; [3]Dept. of Intrnl. Medicine, Section of Endocrinol., Yale Univ., New Haven, CT

Abstract: Intracerebral hemorrhage (ICH) accounts for about 15% of all strokes and has the highest mortality rates among strokes with up to 50% within 30 days after the insult. While direct tissue destruction strikes immediately, secondary injury including perihematoma edema formation, inflammation, and cell death occurs days and weeks following the hemorrhagic event. Hemoglobin and its breakdown product hemin induce toxicity, but it remains unclear how cells die following ICH. The aim of this study was to identify the underlying cell death mechanisms at a pharmacological, biochemical, and morphological level.

We systematically investigated cell death mechanisms after treatment of primary immature cortical neurons with 1.5μM hemoglobin or 100μM hemin. Twenty pharmacological inhibitors of different cell death pathways were studied and cell viability assessed. We confirmed the identified cell death mechanisms using biochemical markers with western blotting and RT-PCR in vitro and in vivo (collagenase ICH model in mice). To assess the morphological phenotype of cell death, we performed electron microscopy determining the number of cells per cell death morphology and mitochondria size.

We found that pharmacological inhibitors of ferroptosis – deferoxamine, N-acteylcysteine, the water-soluble analog of vitamin E, trolox, and U0126 – protected against hemoglobin- and hemin-induced toxicity. Furthermore, necrostatin-1, an inhibitor of necroptosis, also reduced cell death dose-dependently. In contrast, inhibiting caspase-dependent apoptosis, calpains, cathephins, protein or mRNA synthesis, autophagy or mitophagy did not increase cell viability. We then showed that phosphorylation of extracellular signal-regulated kinase 1/2, a marker for ferroptosis, as well as gene expression of receptor-interacting protein 1 and 3, a marker for necroptosis were increased following ICH in vitro and in vivo. Using electron microscopy, we identified that hemin induced a necrotic phenotype with loss of membrane integrity and swelling of organelles, but no shrunken mitochondria characteristic for ferroptosis. To be noted, we did not detect any apoptotic bodies in hemin-treated cells.

Our results suggest that iron overload caused by hemorrhagic stroke in vitro shares features of ferroptotic and necroptotic cell death, but not caspase-dependent apoptosis or autophagy. Distinct mechanisms of cell death imply different therapeutic approaches. Current studies are exploring the relevance of these in vitro findings to rodent models of hemorrhagic stroke.

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Poster

605. Neuroprotective Mechanisms: Regeneration and Therapies

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Title: The pharmacological occupancy of the aryl hydrocarbon receptor and its effects on the kynurenine pathway

Authors: *L. G. GARCIA[1], R. CASTAÑEDA-ARELLANO[1], F. PEREZ-SEVERIANO[2], D. GONZÁLEZ-ESQUIVEL[2], R. O. GONZÁLEZ[3], G. ELIZONDO[1], J. SEGOVIA-VILA[1]; [1]CINVESTAV, D.F., Mexico, Mexico; [2]Inst. Nacional de Neurología y Neurocirugía, Mexico City, Mexico; [3]Univ. Autónoma Metropolitana, Mexico City, Mexico

Abstract: L-kynurenine (Kyn) is a key element of the tryptophan metabolism; it is enzymatically converted by kynurenine aminotransferase II (KAT II) to kynurenic acid (KYNA), which acts as an antagonist to the NMDA receptor-glycine site. Kyn is also an endogenous ligand of the aryl hydrocarbon receptor (Ahr), a transcription factor that regulates the expression of various genes. Interestingly, KYNA levels are reduced in several brain regions of Huntington's disease patients. Previously, we reported that KYNA levels are increased in the brains of *Ahr*-null mice and this induces a neuroprotective effect against neurotoxic insults. Based on these results, we decided to determine whether the pharmacologically- induced occupancy of Ahr had effects on KYNA bioavailability. We used TCDD, a powerful Ahr agonist and resveratrol an antagonist of the Ahr, to assess their effects on cerebral KYNA levels. TCDD was used in a single acute dose because of its toxic side effects, whereas resveratrol was administrated for six weeks. We found that both TCDD and resveratrol increased the striatal levels of KYNA in $Ahr^{+/+}$ mice, whereas it had no effect on $Ahr^{-/-}$ mice, indicating that increased KYNA is a consequence of occupying the Ahr. This strongly suggests that modulating Ahr activity could be neuroprotective. Therefore, we injected quinolinic acid (QUIN) into the striatum of both $Ahr^{+/+}$ and $Ahr^{-/-}$ mice previously treated with resveratrol or vehicle, and observed a neuroprotective effect in resveratrol-treated mice, presumably caused by increased KYNA. These data suggest that the pharmacological occupancy of Ahr could be an interesting therapeutic alternative to protect neurons.

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Poster

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Title: Gold Nanoparticles (AuNPs 40 nm) aggravate whole body hyperthermia induced blood-brain barrier breakdown, edema formation and cellular injuries. Neuroprotective effects of nanowired cerebrolysin

Authors: *P. K. MENON[1], A. SHARMA[2], D. F. MURESANU[3], J. V. LAFUENTE[4], A. OZKIZILCIK[5], R. PATNAIK[7], A. NOZARI[8], H. MOESSLER[9], R. TIAN[6], H. S. SHARMA[2]; [1]Banaras Hindu Univ., Varnasi, India; [2]Surgical Sciences, Anesthesiol. & Intensive Care Med., Uppsala Univ. Hosp., Uppsala, Sweden; [3]Clin. Neurosciences, Univ. of Med. & Pharm., Cluj-Napoca, Romania; [4]Neurosciences, Univ. of Basque Country, Bilbao, Spain; [5]Biomed. Engin., [6]Chem. & Biochem., Univ. of Arkansas, Fayetteville, AR; [7]Biomaterials, Biomed. Engin., Indian Inst. of Technology, Banaras Hindu Univ., Varanasi, India; [8]Anesthesiol. & Critical Care Ctr., Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA; [9]Drug Discovery & Develop., Ever Neuro Pharma, Oberburgau, Austria

Abstract: Gold Nanoparticles (AuNPs) are used for treatment of cancer with whole body hyperthermia (WBH) therapy. Furthermore, nanodelivery of drugs using AuNPs for the treatment of Alzheimer's disease is also suggested. However, the safety and efficacy of AuNPs with regard to cellular toxicity is still not well known. Previous experiments from our laboratory showed that AuNPs depending on their sizes and route of administration could induce profound neurotoxicity in normal rats. Thus, 5 and 10 nm AuNPs induced blood-brain barrier (BBB) breakdown, edema formation and neuronal and glial cellular changes. However, 40-50 nm AuNPs in identical doses did not induce any apparent brain pathology. Interestingly, acute WBH

(at 38° C for 4 h) alone induces BBB breakdown, edema formation and cellular injury in healthy. However, repeated WBH for 1 or 2 h daily for 1 week at 38 °C did not result in brain pathology. Thus, it would be interesting to know weather a combination of AuNPs in acute or repeated WBH could further aggravate brain damage in healthy animals.
Male Wistar rats (age 20 to 25 weeks) were subjected to WBH at 38° C in a Biological Oxygen Demand (BOD) incubator (relative humidity 45-47 %; Wind velocity 20-26 cm/sec) either for 4 h or repeated heat exposure for 1 h daily for 1 week. In separate group of rats AuNPs (ca. 50 nm) was administered (5 mg/kg, i.p.) once daily for 1 week subjected to WBH either for 4 h or repeated 1 h heat exposure daily for 1 week. On the 8th day, BBB breakdown to Evans blue albumin (EBA) and radioiodine ($^{[131]}$-Iodine) and brain edema formation using water content was examined in several brain regions. Neuronal changes were examined using Nissl or H&E stain and glial cell activation was demined by immunohistochemistry of glial fibrillary acidic protein (GFAP) immunoreactivity.
Rats treated with AuNPs alone did not exhibit brain pathology after 1 week. However AuNPs intoxicated rats subjected to 4 h WBH exhibited 2-to 4-fold increase in BBB breakdown, 1.5 to 2-fold higher brain swelling and 2- to 4-folds greater increase in neuronal and glial cell injuries. On the other hand, AuNPs did not exhibit brain pathology in repeated WBH rats up to 5 days. However, on the 8th day marked increase in BBB breakdown, edema formation and cellular injuries were seen in the cerebral cortex, hippocampus, cerebellum and brainstem. Interestingly, treatment with TiO2-nanowired cerebrolysin (2.5 ml/kg, i.v.) 2 h after a 4 h WBH session, or once daily in repeated WBH group significantly prevented brain pathology on the 8th day in AuNPs treated animals. These observations are the first to point out that cerebrolysin could be used as an adjuvant therapy with AuNPs in WBH for acute or chronic cancer therapy.

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Poster

605. Neuroprotective Mechanisms: Regeneration and Therapies

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Potential protective effect of dexmedetomidine on ketamine-induced damage in embryonic neural stem cells

Authors: *Q. YIN, F. LIU, S. LIU, M. G. PAULE, C. WANG;
Div. of Neurotoxicology, Natl. Ctr. for Toxicological Research, FDA, Jefferson, AR

Abstract: Ketamine, an antagonist of *N*-methyl-D-aspartate (NMDA) receptors in the brain, is a commonly used pediatric general anesthetic. Evidence that ketamine can induce neural damage especially in the developing brain has been widely accumulating. Dexmedetomidine (Dex), a sedative and analgesic, is a selective agonist of the α_2-adrenergic receptor. It has been proposed that Dex is capable of ameliorating the neurotoxic effects of other general anesthetics but the underlying mechanisms contributing to this protection are still unclear. Embryonic neural stem cells (NSCs) were harvested from GD14 rat fetuses, cultured in neural stem cell growth medium for seven days, then exposed to different concentration (0.1nM,1nM,10nM,100nM,1μM,10 μM and 100 μM) of Dex with or without ketamine (10 μM) for 3, 6 or 24 hours. Mitochondrial viability, LDH release, and cellular reactive oxygen species (ROS) were evaluated using MTT, LDH and ROS assays, respectively. Our preliminary data indicate that no significant toxicological effects of Dex were observed in these embryonic NSCs: While the potential protective effects of Dex are still under investigation, the early dose-response and time-course data suggest that Dex may serve as protective agent against ketamine-induced neurotoxicity.

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Poster

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Program#/Poster#: 605.04/X1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Metallothioneins: new therapeutic targets in lysosomal storage disorders

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Abstract: Lysosomal Storage Disorders (LSDs) comprise more than 40 diseases, mostly characterized by neurodegeneration. For many LSDs current therapies are not effective in treating the neurologic symptoms, due to the impossibility to target the brain. The family of Metallothioneins (MTs) has been described as neuroprotective therapeutic tools for acute and chronic brain diseases acting mostly as anti-oxidant, but so far they have never been proposed for

the treatment of LSDs. We have recently showed that MT family is a reliable and sensitive marker for LSDs, being highly expressed in the peripheral circulation and more importantly in brains of patients and mice affected by LSDs. Moreover we demonstrated that in LSD patients and animal models MTs decrease to pre-symptomatic levels after treatment. All together these observations suggest a putative reactive and protective role of MTs that still needs to be further dissected. Aim of our work is to investigate the therapeutic use of MTs for alleviating neurologic damage in LSDs. To assess the benefit of constitutively high levels of MTs on LSD background, the mouse model of Globoid Cell Leukodystrophy (GLD), affected by a severe and rapidly progressive brain disease, was cross-bred with a transgenic mouse over-expressing MT1 in all tissues. Interestingly enough, the survival of MT1-GLD transgenic mice was slightly increased as compared to normal affected GLD mice. When dissecting the consequences of MT over expression in GLD mice, the most profound effect we observed was a rescue of Purkinje cells from degeneration and apoptosis. This effect was associated to i) a change of microglia phenotype towards an anti-inflammatory status with an increase of Arginase1 and CD206 expression paralleled by a decrease of IL1β and TNFα expression and ii) a reduction of oxidative stress in microglia cells as shown by quantification of ROS levels in these cells. The direct correlation of these events is currently under investigation, but we may speculate on the key anti-apoptotic and anti-oxidative role of MTs in protecting Purkinje neurons, as also suggested by a transcriptome analysis on cerebella of our models in which we further detailed the involvement of these pathways. We are now testing the same MT-overexpressing strategy in Late Infantile Neuronal Ceroid Lipofuscionosis (LINCL) mouse model, known to be affected by severe neurodegeneration. We have currently under behavioural evaluation MT-NCL mice that show a delayed disease progression as compared to NCL mice, resulting also in an improved survival. Overall, these data may pave the way for exploiting MT features for a therapeutic approach for neurodegenerative LSDs.

Disclosures: **E. Cavalca:** None. **M. Cesani:** None. **M. Peviani:** None. **D. Pellin:** None. **C. Di Serio:** None. **A. Biffi:** None.

Poster

605. Neuroprotective Mechanisms: Regeneration and Therapies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 605.05/DP05 (Dynamic Poster)

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Dendrite versus axon regeneration in central nervous system repair: which way to grow?

Authors: *L. MOONS[1], A. BECKERS[2], L. ANDRIES[2], J. VAN HOUCKE[2], I. BOLLAERTS[2], I. VAN HOVE[2], L. DE GROEF[2], K. LEMMENS[2];
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Abstract: Despite intensive research, successful regeneration and subsequent functional recovery of the injured or diseased mammalian central nervous system (CNS) can still not be evoked. One essential component of the neuronal circuitry might have been overlooked for decades: the dendrites.
During development of the retinofugal system, there is a distinct temporal window for axon *versus* dendrite outgrowth, in which axogenesis precedes dendritogenesis. Whether this recapitulates in the damaged adult CNS remains elusive, but loss of dendritic arborization and connectivity is being recognized as one of the first stages of neurodegeneration, also in glaucoma. Notably, this immediate retraction of dendrites after optic nerve damage might reflect an attempt to repeat the ordered developmental program in adult organisms. As axonal regeneration cannot yet be induced successfully in mammals, we investigated this intriguing hypothesis in the spontaneously regenerating zebrafish, which provides a unique model in the quest for crucial molecules and signaling pathways contributing to successful CNS regeneration. Using a combination of state-of-the-art molecular, biochemical and morphological tools, we were able to show that similar as in mammals, dendritic shrinkage occurs after optic nerve crush (ONC) in adult zebrafish. However, this is only temporal here and quickly followed by a recovery phase in which dendrites regain their length, morphology and synaptic connectivity. Strikingly, pharmacological approaches that counteract this temporal dendritic pruning, result in a reduced RGC axonal regrowth and optic tectum reinnervation. A detailed spatiotemporal characterization of the axonal and dendritic regeneration processes in fish subjected to ONC indeed revealed that axonal and dendritic outgrowth are antagonistic processes and one occurs at the expense of the other.
Overall, our findings disclosed that transient RGC dendritic shrinkage is necessary to induce spontaneous axonal regeneration in the adult zebrafish, and thus that the orderly developmental program of neurite outgrowth repeats. Currently ongoing investigations aim to shed light on how dendritic shrinkage/remodeling contributes to axonal regeneration in the injured zebrafish retinotectal system and to elucidate key molecular signals that regulate these processes, thereby contributing to the development of novel regenerative strategies promoting repair in human age-related neurodegenerative diseases such as glaucoma, but also Alzheimer's and Parkinson's disease.

Disclosures: L. Moons: None. **A. Beckers:** None. **L. Andries:** None. **J. Van houcke:** None. **I. Bollaerts:** None. **I. Van Hove:** None. **L. De Groef:** None. **K. Lemmens:** None.

Poster

605. Neuroprotective Mechanisms: Regeneration and Therapies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 605.06/X2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: National Natural Science Foundation of China(31400942)

the National Program on Key Basic Research Project of China(973 Program,2014CB542205)

Title: Protective mechanisms of Lycium barbarum polysaccharide in a murine hepatic encephalopathy model

Authors: *L. JINGJING[1,2], L. HUANG[2], Y. LV[2], C. REN[2], J. XIAO[2], K.-F. SO[3];
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Abstract: Hepatic encephalopathy (HE) is a systematic dysfunction syndrome with metabolic disorders of the central nervous system (CNS) which is caused by severe liver damages and/or portosystemic shunt. HE has complicated pathological mechanisms and can seriously threatens patients' life. Lycium barbarum polysaccharide (LBP) is a biologically active extract which can regulate immune- and neuroendocrine-functions. In this study, we aimed to investigate the protective mechanisms of LBP in a murine HE model. We established this model by an intraperitoneal single dose injection of 400 mg/kg thioacetamide (TAA) in C57BL/6 mice. LBP (5 mg/kg) was intragastrically administered for once a day. Behavioral, biochemical and molecular tests were used to investigate the mechanisms. We also conducted cell experiment by culturing the rat normal hepatocyte line BRL-3A to examine the protective effect of LBP. We found that LBP significantly improved liver functions and cognitive performance of mice by suppressing hepatic/raphe/hypothalamus apoptosis, necrosis, oxidative stress and inflammation. MAPK and autophagic pathways played critical roles in those processes. In line with previous findings, amelioration of hyperammonemia contributed to the neuronal/hepatic protective properties of LBP. Furthermore, by using BRL-3A cell model, we confirmed that increased serum levels of pro-inflammatory cytokines and ammonia secreted from damaged liver were the major causes of brain damages. In conclusion, we proposed that LBP is a promising nutraceutical supplement for the daily prevention and clinical amelioration of HE.

Disclosures: **L. Jingjing:** None. **L. Huang:** None. **Y. Lv:** None. **C. Ren:** None. **J. Xiao:** None. **K. So:** None.

Poster

605. Neuroprotective Mechanisms: Regeneration and Therapies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 605.07/X3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: College OF Graduate studies, Kuwait University

Title: Thymoquinone protects neurons in intracerebro-ventricular kainic acid model of temporal lobe epilepsy by enhancing the gliosis and BDNF and VEGF levels

Authors: *H. UPTON, M. S. RAO;
Dept. of Anat., Kuwait Univ., Jabriya, Kuwait

Abstract: Temporal lobe epilepsy (TLE) is the most common type of epilepsy among other types of epilepsies. During the early phase after status epilepticus, there will be a significant neuronal death, gliosis and increase in neurogenesis in the dentate gyrus (DG). A few recent studies and our earlier studies have indicated the neuroprotective role of thymoquinone (TQ), the active component of nigella sativa oil in intracerebro-ventricular kainic acid (ICV-KA) model of temporal lobe epilepsy. TQ is shown to be a promising anticonvulsant for epilepsy treatment. Although thymoquinone, showed to be effective in restraining ICV-KA induced epileptic changes in the hippocampus and hippocampal neurogenesis, its mechanisms of actions have not previously been studied. Objective of the present study was to investigate the mechanisms of actions of thymoquinone (TQ) in neuroprotection in intra-cerebro-ventricular kainic acid (ICV-KA) model of temporal lobe epilepsy in young rats. Male Wistar rats (4months old) were divided into three groups: (1) Normal control (NC, n=12), (2) lesion only (LO, n=12), and (3) lesion + thymoquinone (L+TQ, n=12). Rats in the control group remained undisturbed. Rats in the lesion only group received a kainic acid lesion bilaterally in the hippocampus (0.5µg/ventricle). Rats in the lesion + Thymoquinone group were treated with thymoquinone (10mg/kg) intraperitoneally, 3 hours before lesion and daily thereafter for four days. On 5th post-lesion day rats in all groups were anesthetized and perfused with 4% paraformaldehyde. Brains were dissected and processed for cresyl violet, Flurojade-B staining and NeuN, OX-42 and GFAP immunostaining. Fresh hippocampal tissues were dissected and processed for Western blot analysis (GFAP) and analysis of BDNF and VEGF. Results of the study showed significantly decreased neurodegeneration, in the KA+TQ treated rats compared to lesion only group. Astrocyte number and GFAP content and microglial cells were significantly increased in TQ treated group ($p<0.01$) compared to control and lesion only group. Further, TQ treatment also increased the levels of BDNF and VEGF in the hippocampus ($p<0.01$) in TQ treated group. We conclude that TQ protects the neurons by increasing the astrogliosis and increasing the release of neurotrophic factors.

Disclosures: H. Upton: None. **M.S. Rao:** None.

Poster

605. Neuroprotective Mechanisms: Regeneration and Therapies

Location: Halls B-H

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Program#/Poster#: 605.08/X4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Korean Health Technology R&D Project (M-SC, A120476), Ministry of Health & Welfare, Republic of Korea

Basic Science Research Program through the National Research Foundation of Korea (M-SC, NRF-2015R1D1A1A01056950)

Title: Insulin-like growth factor binding protein 6 released from human mesenchymal stem cells confers neuronal protection

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Abstract: Human bone marrow-derived mesenchymal stem cells (hMSCs) are a desirable cell source for cell-based therapy to treat nervous system injuries due to their ability to differentiate into specific cell types. In addition to their multipotency, hMSCs render the tissue microenvironment more favorable for tissue repair by secreting various growth factors. Our previous study demonstrated that hMSCs secrete many growth factors, including several insulin-like growth factor binding proteins (IGFBPs). Among them, IGFBP6 with high-affinity binding to IGF-2 inhibits the growth of IGF-2-dependent tumors by inhibiting IGF-2. However, relatively little is known about the function of IGFBP6 in the nervous system. Here, we elucidated the protective effects of IGFBP6 secreted by hMSCs on H_2O_2-injured primary cortical neuron cultures and LPC-injured organotypic spinal cord slice cultures. Treatment of H_2O_2-injured cortical neurons with conditioned media from hMSCs (hMSC-CM) increased phosphorylation of Akt and reduced both cell death and nuclear translocation of Bax. A blocking antibody against IGFBP6 abolished this hMSC-CM-mediated neuroprotective effect in both injured cortical neuron cultures and spinal cord slice cultures. In addition, treatment with cyclolignan PPP, an inhibitor of the IGF-1 receptor IGF-1R, significantly inhibited neuronal protection by hMSC-CM. These findings demonstrate that hMSC-CM-mediated neuroprotection is attributed to IGF-1R-mediated signaling by IGFBP6. Our study provides insight into the mechanism by which hMSC administration may promote recovery from nerve injury.

Disclosures: **M. Chang:** None. **H. Jeon:** None. **J. Shin:** None. **J. Park:** None.

Poster

605. Neuroprotective Mechanisms: Regeneration and Therapies

Location: Halls B-H

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Program#/Poster#: 605.09/X5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NSF grant IOS1353724

NJCBIR fellowship CBIRFEL002

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Title: The role of Brain-Derived Neurotrophic Factor in the protection of hippocampal neuron networks after glutamate-induced excitotoxicity

Authors: *K. M. O'NEILL[1,2], B. L. FIRESTEIN[1];
[1]Cell Biol. & Neurosci., [2]Biomed. Engin., Rutgers, the State Univ. of New Jersey, Piscataway, NJ

Abstract: In traumatic brain injury (TBI), the physical injury causes mechanical damage to nervous tissue and activates a chemical cascade, resulting in glutamate-induced excitotoxicity (GIE). Despite improved knowledge of the mechanisms of TBI, there is still no agreed-upon treatment for GIE. In particular, N-methyl-D-aspartate receptors (NMDARs) have been implicated in GIE: overactivation of NMDARs leads to excessive levels of intracellular calcium levels, increased rate of cellular events, and excitotoxicity. However, attempts to use NMDAR antagonists as a therapy for TBI have failed. Thus, we are employing a different approach by using brain-derived neurotrophic factor (BDNF) as a neuroprotective agent because it is a pro-survival factor and is overexpressed in neurons that survive injuries, such as ischemia. BDNF prevents chromatin condensation following excitotoxic insult and preserves protein levels of axonal, dendritic, and excitatory synaptic markers. While valuable, these studies lack a functional component to demonstrate that BDNF ensures that neuronal activity is still properly integrated at the network level. To record network activity, dissociated hippocampal neurons from E18 rat embryos are cultured on 64-electrode microelectrode arrays (MEAs). MEAs non-invasively record local field potentials from the group of neurons contacting each electrode. Network parameters, such as spiking (overall) activity, bursting (organized) activity, and synchronization, are collected for the entire culture as well as each electrode. Comparisons are made over time to determine the effects of injury and recovery. Previously, our laboratory used MEAs to examine changes in network activity of cortical neurons after excitotoxic injury with and without drug treatment. The current work suggests that hippocampal neurons are more

sensitive to glutamate-induced injury than cortical neurons: injuring hippocampal neuron cultures for 30 min with 100 μM glutamate abolishes bursting activity at 24 hr after injury, whereas injury with 30 μM has no effect and 60 μM trends toward decreasing bursting. Therefore, we used 30 μM (minimal injury) and 60 μM (moderate injury) for all experiments. For recovery treatments, 50 ng/mL BDNF was chosen because treatment with this concentration, and not 25 ng/mL, significantly increases synchronization. Finally, our results demonstrate that BDNF trends toward conferring protection, as measured by synchronization, to hippocampal networks at 24 hr after injury with 30 μM glutamate and at 72 hr after injury with 60 μM glutamate. These results suggest an optimal window for BDNF treatment after excitotoxic injury associated with TBI.

Disclosures: K.M. O'Neill: None. **B.L. Firestein:** None.

Poster

605. Neuroprotective Mechanisms: Regeneration and Therapies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 605.10/X6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: The effects of exercise pattern and intensity on Flk-1 and Flt-1 expression in the hippocampus

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

Abstract: Exercise produces neurovascular changes in the brain, which can be neuroprotective and support ischemic stroke recovery (Austin et al., 2014). Exercise exerts these protective effects, in part, by increasing angiogenesis, the growth of new capillaries from preexisting blood vessels (Kerr & Swain, 2011). Increased angiogenesis, following ischemic stroke, highly correlates with improved functional outcomes in animal models and human patients (Hayashi et al., 2003). Understanding how exercise pattern and intensity influence angiogenesis could lead to improved exercise rehabilitation programs following ischemic stroke. One critical factor involved in the regulation of angiogenesis is vascular endothelial growth factor (VEGF) and its receptors Flk-1 and Flt-1. Although exercise is generally found to be beneficial, there are wide variations in the exercise regimens used across experiments. This study standardized these variations. In this study, sixty male rats were divided into six equal groups: Voluntary Exercise-Unrestricted (VX-U), Voluntary Exercise- High Intensity (VX-H), Voluntary Exercise- Low Intensity (VX-L), Forced Exercise- High Intensity (FX-H), Forced Exercise- Low Intensity (FX-

L), and Inactive Control (IC). VX-U, VX-H, and VX-L were be placed in cages with attached running wheels. VX-U remained in these cages for the study duration, but VX-H and VX-L were returned to their home cages after reaching 1,000 revolutions (high intensity) and 500 revolutions (low intensity), respectively. FX-H and FX-L were required to run on a motorized wheel at 11m/min. FX-H and FX-L were returned to their home cages after reaching 1,000 and 500 revolutions, respectively. ICs remained in their home cages during the exercise regimens. All animals were sacrificed at the end of the exercise regimens. Exercising animals were then compared to inactive controls, based on unbiased stereological quantification of Flk-1 and Flt-1 immunohistochemical labeling in the hippocampus. Preliminary findings indicate that voluntary exercise animals, allowed unrestricted access to running wheels, display a greater Flk-1 area fraction in the CA1 region of the hippocampus compared to high intensity forced exercise animals and inactive controls. Animals allowed unrestricted access to running wheels also display a greater Flt-1 area fraction in the DG region of the hippocampus.

Disclosures: M. Stevenson: None. **V.K. Behnke:** None. **V.G. Beltrone:** None. **H.E. Hobson:** None. **R.A. Swain:** None.

Poster

605. Neuroprotective Mechanisms: Regeneration and Therapies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 605.11/X7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Peripheral neurotrophic factors in the regulation of adipose tissue energy expenditure.

Authors: M. BLASZKIEWICZ, E. WOOD, *K. L. TOWNSEND;
Univ. of Maine, Orono, ME

Abstract: In order to maintain proper energy balance and metabolic health, the body must tightly regulate the processes that control energy intake (appetite, food intake, nutrient absorption) as well as energy expenditure (physical activity, basal metabolism, thermogenesis). An important aspect of regulating energy expenditure is the transfer of signals from the brain through sympathetic nerves to activate lipolysis and thermogenesis in white and brown adipose tissues, respectively. Cold-stimulation is able to increase the sympathetic innervation and activation of adipose tissues and thus increase energy expenditure through lipolysis and thermogenesis. The exact mechanisms by which cold (or other stimuli that increase energy expenditure) is able to mediate peripheral nerve plasticity are currently under-investigated and largely unclear. In the current project, we provide new evidence that adipose tissue-resident immune cells, including macrophages, are able to secrete a neurotrophic factor, brain derived

neurotrophic factor (BDNF), in response to cold-stimulation. This release of BDNF likely stimulates sympathetic nerve branching, neurite outgrowth and survival, and synapse formation in order to stimulate energy-expending processes in adipose depots. BDNF SNPs have been associated with human obesity and mice lacking BDNF show extreme energy balance dysregulation. While BDNF is well-studied in the brain, it has been largely uninvestigated in adipose tissues. We have found that deletion of BDNF from the myeloid lineage (including macrophages, using LysM-Cre) results in a severe loss of neural innervation of adipose depots, and a shift in energy balance that leads to increased adipose mass, decreased thermogenesis, and lower energy expenditure. Interestingly, while we hypothesized that loss of BDNF secretion from adipose-resident immune cells would also prevent the process of cold-induced 'browning', or development of brown adipocytes in white adipose depots, we were surprised to observe that LysM-BDNF-KO animals do indeed display browning after cold exposure, but the brown adipocytes are mostly clustered around the vasculature and do not express high levels of uncoupling protein 1 (UCP1), the unique marker for brown adipocytes that confers their thermogenic capacity. In total, our findings support the notion that locally-secreted neurotrophic factors are important for maintaining proper innervation and neural stimulation of energy expending processes in adipose tissues, including the activation of UCP1-mediate thermogenesis.

Disclosures: **M. Blaszkiewicz:** None. **E. Wood:** None. **K.L. Townsend:** None.

Poster

605. Neuroprotective Mechanisms: Regeneration and Therapies

Location: Halls B-H

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Program#/Poster#: 605.12/X8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: P01 AI100263

R01 NS057482

Title: Brain microvasculature defects and Glut1 deficiency syndrome averted by early AAV9 mediated repletion of the Glucose Transporter 1 protein

Authors: C. B. RUEDA[1], M. TANG[1], G. GAO[3], K. E. ENGELSTAD[1], J. MCCONATHY[4], D. C. DE VIVO[1], *U. MONANI[2];
[2]MNC, [1]Columbia Univ., New York, NY; [3]Univ. of Massachusetts Med. Sch., Boston, MA; [4]Univ. of Alabama, Birmingham, AL

Abstract: Glut1 deficiency syndrome (Glut1 DS) is a severely disabling neurodevelopmental disorder caused by haploinsufficiency of the *SLC2A1* gene and thus reduced levels of its translated product, the Glucose Transporter Type 1 (Glut1) protein. Although one consequence of insufficient Glut1 protein - a paucity of brain glucose - is widely recognized, precisely how this condition results in the complex Glut1 DS phenotype is unclear. Here we demonstrate that reduced Glut1 protein has a profoundly deleterious effect on brain angiogenesis and on the maintenance of the cerebral microvasculature, without compromising the blood-brain barrier. Early, AAV9-mediated repletion of the protein in neonatal Glut1 DS model mice ensures the proper development of the microvasculature of the brain and arrests disease evolution. Augmenting the protein in juvenile mutant mice that have already suffered sustained low brain glucose is less effective in establishing normal brain microvasculature, yet reverses mutant brain glucose concentrations and has a pronounced overall therapeutic effect. In contrast, delayed delivery of the protein - to adult mice - neither reverses defects of the brain capillary network nor rescues the Glut1 DS phenotype. Our results link brain dysfunction in Glut1 DS to novel defects of the cerebral microvasculature, a condition that likely upsets the evolving circuitry of the maturing brain. The results, furthermore, identify a limited postnatal therapeutic window for Glut1 DS, yet suggest that timely reinstatement of the Glut1 protein, even under prolonged conditions of profoundly low brain glucose, can prevent disease. Early repletion of the Glut1 protein may thus constitute the most effective treatment yet for Glut1 DS.

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Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 606.01/X9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Ppars modulates the anti-inflammatory effects of melatonin in the secondary events of spinal cord injury.

Authors: *I. PATERNITI, M. CAMPOLO, M. CORDARO, D. IMPELLIZZERI, R. SIRACUSA, E. ESPOSITO, S. CUZZOCREA;
Univ. of Messina, Messina, Italy

Abstract: Melatonin is the principal secretory product of the pineal gland and its role as an immumo-modulator is well established. Recent evidence shows that melatonin is a scavenger of oxyradicals and peroxynitrite and reduces the development of inflammation and tissue injury

events associated with spinal cord trauma. Previous results suggest that Peroxisome proliferator activated receptor alpha- α (PPAR-α), an intracellular transcription factor activated by fatty acids, plays a role in control of secondary inflammatory process associated with spinal cord injury (SCI).
With the aim to characterize the role of PPAR-α in melatonin mediated anti-inflammatory activity, we tested the efficacy of melatonin (30 mg/kg) in an experimental model of spinal cord trauma induced in mice by the application of vascular clips (force of 24 g) to the dura via a four-level T5-T8 laminectomy, and comparing mice lacking PPAR-α (PPAR-αKO) with wild type (WT) mice.
The results obtained indicate that melatonin -mediated anti-inflammatory activity is weakened in PPAR-α KO mice, as compared to WT controls. In particular, melatonin was less effective in PPAR-α KO, compared to WT mice, as evaluated by inhibition of the degree of spinal cord inflammation and tissue injury, neutrophil infiltration, pro-inflammatory cytokine expression, NF-κB activation, inducible nitric-oxide synthase (iNOS) expression. This study indicates that PPAR-α can contribute to the anti-inflammatory activity of melatonin in SCI.

Disclosures: I. Paterniti: None. **M. Campolo:** None. **M. Cordaro:** None. **D. Impellizzeri:** None. **R. Siracusa:** None. **E. Esposito:** None. **S. Cuzzocrea:** None.

Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 606.02/X10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant NS766726

Title: Live imaging of innate immune response in neonates reveals differential activation patterns in brain injury and infection

Authors: M. LALANCETTE-HEBERT[1], G. SOUCY[1], J. FAUSTINO[2], Z. VEXLER[3], *J. KRIZ[1];
[1]Laval Univ., Quebec, QC, Canada; [2]Neurol., Univ. of California, San Francisco, CA; [3]Neurol., Univ. of California, San Francisco, CA, Canada

Abstract: Inflammatory response can be a strong contributor to injury evolution in adult central nervous system. Moreover, in immature brain, neuroinflammation episode, triggered by neonatal stroke or systemic infections, can be responsible for long term neurological deficits. However, the potential role of inflammation and immune cells activation in normal brain development is

less characterized. In order to study the role of microglial cells, a CNS specific immune cell type, we took advantage of our live imaging model, the TLR2-luc-GFP mouse. We follow longitudinally the microglial/TLR2 activation in the brain of live animals from postnatal day 6 (PN6) to adulthood. The spatial expression of TLR2 is very dynamic during the first weeks after birth, initially emerging from the whole brain and become more and more restricted to the olfactory bulb at the end of the first month. Furthermore, the identity of TLR2 expressing cells also change during time. In fact, neurons and microglia express TLR2 in early PN age while 28 days after birth, microglial cell is the major cell type expressing it. These results suggest that the spatiotemporal expression of TLR2 can be determinant in the brain response to diverse inflammatory conditions. To test this hypothesis, we first mimic a systemic infection using i.p. LPS injection at PN6 and PN9. The expression of TLR2 is upregulated in the brain in both PN ages 24 hrs after LPS injection. The cytokines profile correlate with the induction of TLR2 seen by live imaging and most pro-inflammatory cytokines like IL-1β, IL-17 and TNFα are upregulated in both time points. On the other hand, we wanted to compare the inflammatory response of immature brains after LPS injection with a model of sterile inflammation like a stroke. To do so, we use intra-cortical injection of IL-1β on PN6 and PN9 pups and follow the inflammatory response by live imaging. To our surprise, the TLR2 activation is downregulated 24 hrs after injection. Once again, the cytokines profile correlates with this downregulation of TLR2 signal. In fact, the major proinflammatory cytokines are decreased. In both pathological models, the immune reaction seems to be more prononced at PN6 when compared to PN9 indicating that the level of maturity of brain and the immune system can be an important component in the outcome of the damage resolution. Those results clearly show that different inflammatory stimuli can initiate contrasting innate immune reaction in immature brain. A better knowledge of the specific spatiotemporal sequence of events after an injury or infection will allow us to better control and adapt the treatment of neonates to minimise long term neurological handicap.

Disclosures: **M. Lalancette-hebert:** None. **G. Soucy:** None. **J. Faustino:** None. **Z. Vexler:** None. **J. Kriz:** None.

Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

Location: Halls B-H

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Program#/Poster#: 606.03/X11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: MOST 104-2314-B-037-069-

Title: The highly electronegative low density lipoprotein l5 induces the reactive activation and inflammation in bv2 microglia

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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 microglia reactivity is fully unknown and has not been studied. Here, an in vitro cultured model of BV-2 cell, a murine microglia cell line, was used in this study. We treated the cells with lipopolysaccharides (LPS), L5, L1 (an LDL with lesser extent of electronegativity than L5) and oxidized LDL (an artificially synthesized LDL) and assayed for gliotoxicity and inflammation responses, including the release of inflammation mediators, the expression of inducible stress proteins, the induction of apoptosis and cell migration. The results indicated that L5 stimulates BV-2 cell activation, evidenced by the production of nitric oxide (NO), reactive oxygen species, peroxynitrite and TNF-α, and the induction of inflammatory proteins (e.g., inducible NO synthase, cyclooxygenase-2 and hemeoxygenase-1), consequently initiating an inflammation. Moreover, L5 leaded to apoptosis and potentiated cell migration, detected by DAPI staining and wound-filling assay, respectively. These results suggested that L5 itself has the potential to trigger inflammatory responses and cause eventual death in immune cells, like microglia.

Disclosures: **J. Wang:** None. **L. Yu:** None. **C. Lai:** None. **C. Lee:** None.

Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 606.04/X12

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH-NIDCR #DE021888 (OJI)

UMKC School of Graduate Studies Program

UMKC Graduate Assistance Found

Title: Toll-like receptor 4 priming sensitizes macrophages to oxidant-mediated Cox2 gene expression and PGE2 production

Authors: *Y. ZHANG[1], O. J. IGWE[2];
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Abstract: **Background:** In addition to pathogen associated molecular patterns and damage associated molecular patterns, toll-like receptor 4 (TLR4) can be triggered by various environmental factors, e.g. ozone, atmosphere particulate matter, and ionizing radiation etc. Prostaglandin E2 (PGE2) produced from induction of phospholipase A2 (PLA2), cyclooxygenase 2 (Cox2) and membrane-associated PGE sysnthase-1 (mPGES-1) is a potent proinflammatory mediator.
Objective: This study examined the role of TLR4 priming in mediating oxidant-induced activation of Cox 2 and PGE2 production in macrophages
Methods: Potassium peroxychromate (PPC) and peroxynitrite (PN) were used as prooxidant sources and a TLR4-specific agonist LPS-EK was used to prime or sensitize TLR4. We used primary murine peritoneal macrophages (pM) isolated from C57BL/6 mice expressing TLR4 (TLR4-WT) and B6.B10ScN-*Tlr4* mice with complete deletion of TLR4 (TLR4-KO). pM were sensitized for 4 h with 100 ng/ml LPS-EK, then incubated for another 24 h with fresh medium containing PPC or PN. Intracellular reactive oxygen species (iROS) production was visualized and quantified by fluorescence imaging and flow cytometry, respectively. Total antioxidant capacity within cells was quantified by antioxidant assay kit. We used ELISA to quantify PGE2 released into the media following treatments. The levels of mRNA and protein of phospholipase A2 type V, Cox2, and mPGES-1 were determined by real-time PCR and western blot, respectively.
Results: We found that pro-oxidants treatment increased iROS production and decreased total antioxidant capacity in pM derived from TLR4-WT but not in pM derived from TLR4-KO mice suggesting TLR4 acts as sensor of exogenous oxidants. In addition, we found that treatment with oxidant alone induced a limited increase in PGE2 production. In contrast, pM sensitized with LPS-EK and treated with oxidants exhibited robust Cox2 expression and PGE2 production compared with sensitized pM and vehicle control. The expression levels of mRNA and proteins for PLA2 type V, Cox 2 and mPGES-1 were significantly increased. The induction of Cox2 and subsequent PGE2 production was observed in pM derived from TLR4-WT mice but not TLR4-KO mice.
Conclusions: Taken together, TLR4 priming sensitized primary macrophages to oxidant-induced Cox2 expression and PGE2 production. The data provide a potential mechanism by which prooxidants facilitate human disease states (inflammatory processes in the presence of bacterial LPS). The data further confirms the potential threat posed by the ubiquitous bacterial LPS and oxidative stress.

Disclosures: **Y. Zhang:** None. **O.J. Igwe:** None.

Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: FNS; grant number: FN31003A-127177

Title: Tollip modulates the early phase of LPS-induced neuroinflammation

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Abstract: Tollip is a ubiquitous protein, originally described as a modulator of the IL-1R/TLR-NFκB signalling pathway. Although this property has been well-characterized in peripheral cells and despite evidence of its expression into the central nervous system, the role of Tollip in neuroinflammation remains poorly investigated. The present study sought to explore the implication of Tollip in the response to an inflammatory challenge using Tollip knockout (KO) mice. The effects of an intranigral injection of lipopolysaccharide (LPS), a TLR4 receptor agonist, on inflammatory markers have been assessed at transcriptional and protein levels. We show that Tollip deletion had no effect on the basal levels of inflammatory markers, but increased susceptibility to LPS-induced neuroinflammation. Indeed, 6 hours post-injection of LPS at a sub-maximal dose (0.1 μg), a significant increase of TNF-α, IL-1β, IL-6 and IFNγ mRNA was observed in the midbrain of Tollip KO mice, whereas this effect did not reach significance for all the analyzed cytokine in WT mice. In addition, Tollip KO mice displayed a higher LPS-induced inducible NO synthase (iNOS) production, as demonstrated by an increase of iNOS mRNA and iNOS protein immunostaining when compared to LPS-injected WT mice. Finally, Tollip deletion aggravated LPS-induced oxidative and nitrosative damages, as indicated by an increase of 8 oxo-DG and nitro-tyrosine immunostaining, respectively. These effects are associated with an increase of NFκB activation in KO mice evidenced using a AAV reporter viral vectors expressing eGFP mRNA under the control of NFκB-responsive transcriptional sequences. In conclusion, these results show that Tollip KO mice have a global tendency to elicit a higher cytokine production associated with a significant increase of oxidative and nitrosative stress. Unexpectedly, viral vector-mediated Tollip overexpression in neurons of the midbrain did not reveal a decrease but an increase of pro-inflammatory cytokines and NFκB activation.

Altogether, these observations reveal a contribution of Tollip in the regulation of the early phase of the neuroinflammation.

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Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

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Program#/Poster#: 606.06/X14

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Cytokine profile related to treatment response in patients with extraparenchymal neurocysticercosis

Authors: *Y. MARTINEZ LOPEZ[1], J. F. GARCÍA[2], O. HERRERA[2], R. CARRILLO[2], H. JUNG[2], L. ADALID[2], I. GONZÁLEZ[2], A. TOLEDO[2], E. GARCÍA[2], A. FLEURY[2];
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Abstract: Introduction: Neurocysticercosis (NCC) is the most common helminthic infection affecting the human central nervous system. It remains, as a disease of epidemiological importance in developing countries, owing to is associated with high morbidity and disability in productive population. It produces a wide range of neurological (motor, sensory, cognitive, etc.) and psychiatric manifestations requiring recurrent hospitalizations. Regarding treatment, the response is variable depending on the location, number and stage of the parasite. Extraparenchymal location of the parasite causes the most severe presentation of the disease, frequently manifested by intracranial hypertension. In these cases, the lack of response to cysticidal drugs occurs in 30-40 % of the patients. In this research we evaluated whether or not non-response to treatment is associated with any immunological features.
General Objective: Evaluate the relationship between the immunological factors and response to treatment in patients with extraparenchymal neurocysticercosis.
Material and methods: Prospective, longitudinal, comparative and analytical study. 16 patients with definitive diagnosis of vesicular extraparenchymal neurocysticercosis were included. Lymphoproliferation assays using *Taenia solium* antigens were performed on PBMC before and after (1 and 4-6 months) treatment. Proinflammatory cytokines (IL-1B, IL-6 y TNFα) were measured on supernatans and CSF, as well as the concentration of albendazol sulfoxide (ASOX) in sera. Patients were evaluated clinically and radiologically (3D IRM, volumetric measurement

of the cysts). The response to treatment was determinated by the volume cyst index (pre treatment/post-treatment volume cyst). Correlations between the response to treatment and immunological parameters were evaluated. As well as its relation to age, gender and ASOX levels.
Results: 16 patients were included and according to the volume cyst index we classified them as 8 responders and 8 non-responders to treatment. Both groups showed similar demographic characteristics (gender, age) and ASOX levels were not significantly different between groups. We found positive correlations between volume cyst index and proinflammatory cytokines in supernatans (IL-1β pre-treatment) (R=0.52, P= 0.03), IL-1β 1 month after treatment (R=0.49, P= 0.05) and IL-6, 1 month after treatment (R=0.65, P=0.007). The non-responder group had a decreased lymphoproliferative response (P=0-004).
Conclusions: An exacerbated inflammatory state before and after cysticidal treatment is associated with a better response to treatment.

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Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

Location: Halls B-H

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Program#/Poster#: 606.07/X15

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: KOREA NRF 2011-0030049

Title: Basal expression of immune related genes is attenuated in Pu.1 knockout microglia

Authors: T. YOON, S. KIM, A. DAS, J. CHAI, Y. LEE, K. PARK, K. JUNG, *Y.-G. CHAI; Mol. Biol. & Life Sci., Hanyang Univ., Ansan, Korea, Republic of

Abstract: Microglia are tissue resident macrophages which have important roles in immune system in the CNS. Pu.1 is a transcription factor which organizes cell type specific gene expression pattern in myeloid cells including microglia, and closely related to immune response under the inflammatory stimuli. To investigate the regulatory roles of Pu.1 in microglia, we constructed Pu.1 knockout (KO) BV-2 microglial cell line using CRISPR-Cas9 system and performed comparative transcriptomic analysis between wild type and Pu.1 KO BV-2 cells by the use of web-based analysis programs including IPA. The results showed that the expression levels of 176 genes were significantly altered (75 up-regulated and 101 down-regulated; ±1.5

log2-fold change, 0.05 p-value and q-value thresholds) by Pu.1 KO. The functional annotations presented that the majority of down-regulated genes were related to immune responses, and the analyses on cellular functions and gene networks also predicted that cell activation and immune responses would be repressed in Pu.1 KO cells. In addition, ChIP-qPCR results of representative genes obtained from the analyses showed that Pu.1 binding is related to the expression of those genes. In conclusion, through the transcriptomic analyses and following experiments, we reconfirmed the regulatory roles of Pu.1 in immune responses of microglia, and also presented that Pu.1 plays its regulatory role even in resting state microglia.

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Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIAAA AA011605

Title: SH-SY5Y neurons and BV2 microglia show unique neuro-immune responses to ethanol and TLR agonists that are modified by co-culture

Authors: *C. J. LAWRIMORE, F. T. CREWS;
UNC Chapel Hill, Chapel Hill, NC

Abstract: Introduction: Excessive ethanol (EtOH) consumption causes neuroimmune activation, which is linked to neurodegeneration and addiction. While microglia function as the primary macrophage in brain, neurons are emerging as a novel player in innate immune signaling. In order to investigate cell-type-specific immune signaling, neuron-like SH-SY5Y and microglia-like BV2 were treated with EtOH or agonists to TLR3 or TLR4, and cell lysates/media were examined for cytokine expression. To determine whether communication between the two cell types modifies immune signaling, cells were also co-cultured prior to treatments.
Methods: BV2 microglia and SH-SY5Y neurons were treated with either TLR3 agonist PolyI:C (50ug/mL), TLR4 agonist LPS (100ng/mL), or EtOH (100mM) for 24hr. In co-culture groups, BV2 were plated on top of SH-SY5Y using Transwell inserts, allowing for signaling communication without physical contact. Cell lysates and media were analyzed using RT-PCR and ELISA, respectively. Gene expression data is shown as % control for respective cell-type and culture condition.

Results:
SH-SY5Y neurons alone showed no response to LPS. However, when co-cultured with BV2 microglia, LPS increased SH-SY5Y cell mRNA of TNFα, IL-1β, and MCP1 (1763%, 2877%, and 752% respectively, $p<0.05$), and increased MCP1 release in media. PolyIC increased TNFα, IL-1β, and MCP1 (9094%, 47402%, and 3287% respectively), with co-culture potentiating TNFα and IL-1β (48738% and 67144% respectively), while MCP1 displayed a dampened increase (2048%). EtOH had no effect on TNFα or IL-1β, but MCP1 was decreased in cells alone (42% of control) and co-cultured (43%). EtOH increased HMGB1 release and decreased MCP1 release in cells alone, and in co-cultured cells both MCP1 and HMGB1release was decreased.
In BV2 microglia, LPS increased mRNA expression of TNFα IL-1β and MCP1 (440%, 1162%, and 340% respectively, $p<0.05$). PolyIC increased TNFα and IL-1β (725% and 1351% respectively) but not MCP1. EtOH increased TNFα and IL-1β (121% and 184% respectively) and HMGB1 release. Co-culture with SH-SY5Y enhanced LPS-induced TNFα, IL-1β and MCP1 (971%, 15521%, and 1065% respectively). PolyIC, co-culturing increased MCP1 (212%), but dampened TNFα and IL-1β responses (483% and 737%). Co-culturing blocked EtOH-induced TNFα and IL-1β, and decreased MCP1 (33±1.3%) and released HMGB1.
Conclusions: Co-cultured BV2 microglia and SH-SY5Y neurons display altered neuro-immune responses. In the presence of microglia, SH-SY5Y neurons become responsive to LPS. Future experiments will examine mechanisms underlying microglia-neuron communication.

Disclosures: C.J. Lawrimore: None. **F.T. Crews:** None.

Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant GM109086

UW Dept of Anesthesiology

Title: Wakeful EEG correlates of systemic lipopolysaccharide

Authors: *M. I. BANKS[1], M. DARRACQ[2], C. N. MURPHY[3], S. M. GRADY[2], R. D. SANDERS[2];
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Abstract: Introduction: Neuroinflammation is associated with cognitive and behavioral anomalies, but the mechanisms linking inflammation to changes in brain activity are unclear. Lipopolysaccharide (LPS) from gram negative bacteria is a common model for systemic inflammation in experimental animals. In young people, controlled administration of LPS induces an inflammatory state characterized by behavioral lethargy and depression but increased alpha and beta power in the EEG. We sought to model these apparent contrasts in the behavioral and EEG responses by studying LPS-induced changes in brain electrical activity in wakeful young mice.
Methods: All procedures were approved by the UW-Madison Animal Care and Use Committee and conform to APS/NIH guidelines. Six mice (1 male; BL6 background; 12-20 wks old) were instrumented with bilateral parietal and frontal skull screw EEG electrodes under aseptic surgical conditions. After 5-7 days recovery, mice were given sham intraperitoneal (IP) saline injections, and 2 days later IP injections of LPS (125 ug/kg). Animals were tethered to a lightweight headstage during the experiment, but otherwise free to move in the recording chamber. Data were collected for 1 hr prior and 4 hrs post-injection. EEG signals were subjected to spectral analysis and average power computed in the δ (1-4 Hz), θ (4-10Hz), α (10-20 Hz), β (20-30 Hz) and γ (30 – 100Hz) bands during movement defined by EMG activity and confirmed via video recording. Power in each band was normalized to the average total spectral power in the control condition. Statistical comparisons (paired t-tests) were between the post-injection:control ratios of normalized band power for saline versus LPS.
Results: There was little effect of saline injection on relative power in any frequency band (median across animals of ratio to control at one hour after injection = 0.76, 0.96, 0.84, 1.0 & 1.0 for δ, θ, α, β & γ, respectively). By contrast, dramatic and significant increases in α & β band power were observed following injection of LPS (median ratio to control at one hour after injection = 2.4 & 15, respectively; $p<0.05$), while variable increases were observed for δ, θ & γ bands (4.5, 1.8 & 1.2, respectively). The increase in α & β power was rapid, occurring within 0.5 hr of injection, while the variable increases in other bands were delayed.
Conclusions: LPS injection into mice is a promising model for neuroinflammatory effects on brain activity in wakefulness confirmed by spontaneous movement. Our future goal is to investigate the neural circuits underlying the observed changes to inform the neuropsychiatric consequences of acute and chronic illness.

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Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Revalesio Corp. (unrestricted gift)

Behavioral tests subsidized by Harvard HNDC Mouse Neurobehavior Lab.

Title: RNS60 nanobubble treatment reduces inflammation and Abeta plaques in aged APP/PS1dE9 mice

Authors: *Q. SHI[1], B. LIU[1], A. S. M. HUNG[2], K. LE[1], B. CALDARONE[3], S. GHOSH[4], R. WATSON[4], C. A. LEMERE[1];
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Abstract: RNS60 (Revalesio Corp., Tacoma, WA) is generated by electrokinetic modification of normal saline and contains an oxygen-filled nanobubble core surrounded by layers of positive and negative electrical charges or “charge stabilized nanostructures”. Previous studies by others have demonstrated that early treatment with RNS60 promotes anti-inflammatory effects and protects against Alzheimer’s disease (AD)-related neurodegeneration and memory decline in the 5XFAD mouse model (Roy et al., 2014; Modi et al., 2014). Here, we asked whether RNS60 would be beneficial in aged, plaque-rich AD transgenic (Tg) mice. Sixteen month-old male APP/PS1dE9 Tg mice were treated with 300 µl intraperitoneal (i.p.) RNS60 (n=13) or Normal Saline (NS; n=8) 3 days per week for 10 weeks. Wildtype (WT) control littermates were treated with i.p. NS (n=8). At 18.5 months of age, learning and memory were significantly impaired in NS-treated Tg mice in Contextual Fear Conditioning (CFC; learning only) and Water T Maze (WTM) compared to NS-treated WT mice. RNS60 treatment resulted in a modest increase in freezing time during CFC training (p=0.059; shock 2) compared to NS-treated Tg mice but had no effect on acquisition or reversal learning in the WTM in the aged mice. RNS60 treatment led to a significant reduction in Aβ42 plaque immunoreactivity in cortex and hippocampus but no change in Aβ levels by ELISA compared to NS-treated Tg mice. Longitudinal ^{18}F-GE180 TSPO microPET imaging before and after treatment showed reduced glial activation in RNS60- vs. NS-treated Tg mice. Immunoreactivity with multiple microglia/macrophage markers was significantly reduced in the hippocampus of RNS60-treated Tg mice compared to NS-treated Tg mice, while astrocyte staining was non-significantly reduced. RNS60 treatment reduced cerebral pro-inflammatory proteins, TNFα and iNOS, and increased anti-inflammatory proteins, IL-10

and YM-1, in RNS60-treated Tg mice compared to NS-treated Tg mice. Hippocampal synaptic puncta density and synaptic protein levels were modestly but significantly increased in RNS60-treated Tg mice compared to NS-treated Tg controls but were significantly lower than NS-treated WT mice. Mature-BDNF, p-CREB and p-ERK, but not ERK1/2 levels, were significantly increased in RNS60-treated Tg mice compared to NS-treated Tg mice. Phospho-GSK3β was increased in RNS60-treated Tg mice, indicating reduced GSK3β kinase activity. In conclusion, RNS60 treatment in aged, plaque-rich AD mice reduced inflammation and plaque load, and modestly improved learning but not memory. This suggests the potential for better recovery with earlier treatment.

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Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

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Program#/Poster#: 606.11/Y1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: SFI Grant SFI/IA/1537

Title: TLX as a protective modulator against IL-1β-induced impairment in hippocampal neurosphere growth

Authors: ***C. O LEIME**[1], J. F. CRYAN[1,2], Y. M. NOLAN[1];
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Abstract: Hippocampal neurogenesis is the process by which new neurons are produced from neural stem cells (NSCs) and is involved in some forms of memory and emotion regulation. Sustained proliferation of NSCs within the hippocampus is a key factor in maintaining normal hippocampal neurogenesis. The pro-inflammatory cytokine IL-1β is an important mediator of neuroinflammation and is elevated in conditions associated with hippocampal dysfunction such as Alzheimer's disease and major depression. Evidence now demonstrates that IL-1β suppresses the proliferation of NSCs. TLX is an orphan nuclear receptor known to regulate NSC proliferation and it has been recently shown that IL-1β reduces TLX expression in proliferating NSCs. We investigated whether a restoration of TLX expression in hippocampal NSCs can prevent the anti-proliferative effects of IL-1β on these cells. Embryonic day 18 rat hippocampal neurospheres were transfected with a lentiviral vector (LV) overexpressing either TLX (OEX) or

Green Fluorescent Protein (GFP) as a control. The formation and growth of the neurospheres were measured in the presence or absence of IL-1β (recombinant protein) for 5 days *in vitro* (DIV). While IL-1β did not impair neurosphere formation it significantly impaired neurosphere growth after 3DIV ($p<0.05$). Early results indicate that TLX overexpression (in the absence of IL-1β) increased neurosphere growth compared to control treatment. Moreover, overexpression of TLX also attenuated the IL-1β-induced reduction in neurosphere growth. This effect appears to be TLX specific as this attenuation is not observed in the LV-GFP-treated neurospheres in response to IL-1β. Analysis is currently being carried out to assess the effects of IL-β and TLX(OEX) on the cellular phenotypes in neurospheres and on their proliferative capacity.

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Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Multiple Sclerosis Society of Canada

Brain Canada

Heart and Stroke Foundation

Killam Laureates

NSERC

endMS Research and Training Network

Title: Kinematic analyses of gait deficits in a mouse model of multiple sclerosis: Alterations in ankle movement predict the degree of spinal cord injury

Authors: *M. FIANDER[1], N. STIFANI[4], T. AKAY[2], G. S. ROBERTSON[3];
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Abstract: Experimental autoimmune encephalomyelitis (EAE) is the most widely used animal model for multiple sclerosis (MS). Symptoms of EAE resemble those observed in MS patients, including loss of motor coordination and paralysis. The primary behavioural measure of EAE disease severity is a 5-10 point scale that reflects qualitative aspects of neurological function.

This ordinal scale provides very limited information about the extent and specific nature of motor disabilities exhibited by EAE mice. In view of these limitations, we compared three methods of assessing motor function in EAE mice: clinical scoring, rotarod and kinematic gait analysis. We have found that rotarod performance and clinical scores were strongly correlated during the acute phase of EAE (15 days post-immunization; dpi; r=-0.8), however, the strength of this correlation weakened at 22 and 29 dpi (r=-0.33 and r=-0.44, respectively). These findings indicate clinical scores are unable to accurately measure the recovery of motor function from peak disease (15 dpi) to chronic EAE (22 and 29 dpi) and that mice with similar clinical scores often vary substantially in their rotarod performance. To assess motor dysfunction from a biomechanical perspective, kinematic gait analysis was performed. Briefly, a high-speed camera that captured 250 frames per second was used to record mice walking on a treadmill. Kinematic analysis of sagittal joint angles (hip, knee and ankle) was then performed on these recordings. There were a variety of changes in the kinematic parameters of EAE mice that varied substantially between animals. In order to simplify the kinematic data, we calculated the root mean square (RMS) of the difference in joint angles between baseline and post-immunization time points for EAE mice. The RMS difference of the ankle and knee joints sensitively detected changes in EAE mice and correlated well with other behavioural measures of disease severity. We then correlated clinical score, rotarod performance and RMS difference of the hip, knee and ankle joints with white matter loss in the spinal cord. Clinical scores and rotarod performance only weakly correlated with white matter loss (r=0.38 and r=-0.43, respectively). By contrast, the RMS difference for the ankle correlated remarkably well with white matter loss (r=0.95) indicating that changes in ankle kinematics are exquisitely sensitive to white matter damage in the spinal cord. These findings indicate kinematic gait analysis is a highly sensitive method for assessing neurological deficits in EAE mice that should facilitate the identification of treatments which promote functional recovery in MS.

Disclosures: **M. Fiander:** None. **N. Stifani:** None. **T. Akay:** None. **G.S. Robertson:** None.

Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Weston Brain Institute

Title: Mri-guided focused ultrasound gene delivery to the brain using an astrocyte-specific promoter in a mouse model of alzheimer’s disease

Authors: *D. WEBER-ADRIAN[1], J. W. Y. CHAN[1], J. SILBURT[1], Z. NOROOZIAN[1], K. SHAH[3], A. BURGESS[2], S. RIDEOUT[2], S. KÜGLER[4], K. HYNYNEN[2], I. AUBERT[1]; [1]Brain Sci. Research, Biol. Sci., [2]Physical Sci., Sunnybrook Res. Inst., Toronto, ON, Canada; [3]Inst. of Med. Sci., Univ. of Toronto, Toronto, ON, Canada; [4]Neurol., Georg-August-Universität Göttingen, Göttingen, Germany

Abstract: Previous studies have demonstrated an increase in glial fibrillary acidic protein (GFAP) expression surrounding amyloid-β (Aβ) plaques in a mouse model of amyloidosis. Harnessing the GFAP promoter to strongly express a transgene in response to Aβ pathology may provide a useful strategy for gene therapy for Alzheimer's disease (AD). Gene therapy offers promise for the treatment of AD; however, the blood brain barrier (BBB) is a challenge for non-surgical delivery of transgenes to the brain. Here, MRI-guided focused ultrasound (MRIgFUS) is used to mediate delivery of an adeno-associated virus (AAV) gene construct from the blood to the brain using a cell-specific GFAP promoter. We hypothesize that AAV-mediated gene delivery under control of the GFAP promoter will result in enhanced transgene expression within the vicinity of Aβ plaque, in which reactive astrogliosis is occurring. A transgenic (Tg) mouse model of amyloidosis and wild-type mice were used. MRIgFUS allowed for transient and local permeabilization of the BBB, thereby facilitating AAV delivery from the bloodstream to FUS-targeted cortical and hippocampal regions. Results indicated that the GFAP promoter is selective for gene expression in astrocytes in the central nervous system, but not in the peripheral system. Transgene expression in Tg animals was compared between FUS-targeted brain regions with and without Aβ plaque, using either a GFAP or ubiquitous promoter. Amount of transgene expression was further compared between Tg and wild-type animals. The GFAP promoter demonstrates a heightened gene expression rate in areas of high Aβ plaque deposition with reactive astrocytes. The next step is to reduce gene expression in the periphery.

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Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Tibial fracture triggers cold allodynia and transient regulation of neuropeptides and BDNF in dorsal root ganglia and hippocampus

Authors: *M. ZHANG[1], S. BARDE[2], T. YANG[4], B. LEI[5], L. I. ERIKSSON[3], J. P. MATHEW[5], K. AKASSOGLOU[6], T. HARKANY[7], T. HÖKFELT[2], N. TERRANDO[5];
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Abstract: Surgery often leads to cognitive impairments, yet the mechanisms whereby systemic injury affects the central nervous system (CNS) remain unclear. Pain is a critical component after surgery and associates with poor functional outcomes. Understanding the role of pain signaling after surgery may help us defining novel interventions for common postoperative complications like delirium and postoperative cognitive dysfunction. Using a murine model of orthopedic surgery we show that tibial fracture selectively triggers cold allodynia and up-regulates nerve injury and inflammatory markers in the primary somatosensory system, including dorsal root ganglia (DRGs) and spinal cord. At 24 h after injury activating transcription factor 3 (ATF3), neuropeptides galanin and neuropeptide Y (NPY), brain-derived neurotrophic factor (BDNF), non-neuronal markers ionized calcium binding adaptor molecule 1 (Iba1), glial fibrillary acidic protein (GFAP) and CX3CR1, were differentially elevated in DRGs. We compared these changes to an established model of complete transaction of the sciatic nerve to evaluate the temporal relationship of these markers. In the CNS we describe a distinct increase in BDNF protein levels in the mossy fibers at 24 h using immunohistochemistry but with no changes in mRNA levels in the granule cells, monitored by both in situ hybridization and RTqPCR. These changes in hippocampal BDNF were paralleled by a reduction in neurogenesis. Our findings indicate that bone fracture in mouse causes, in addition to selective cold allodynia, an unexpected effect on BDNF signaling, selectively in the hippocampal granule cells, resulting in presynaptic accumulation of this growth factor in the mossy fibers, tentatively due to attenuated/blocked release. Overall this apparently novel mechanism may underlie cognitive and mental deficits as often observed after surgery or critical illness.

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Poster

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Ministry of Economy and Competitivity, Government of Spain (PK612884)

Title: Astrocyte-targeted production of either IL6 or IL10 influences peripheral nerve regeneration after facial nerve axotomy

Authors: *G. MANICH[1], N. VILLACAMPA[2], B. ALMOLDA[2], I. CAMPBELL[3], B. GONZÁLEZ[2], B. CASTELLANO[2];
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Abstract: The facial nerve axotomy (FNA) is one of the most well-described models of peripheral nerve injury, and is mainly characterized by retrograde degeneration of motor neurons in the facial nucleus (FN), glial activation and axonal regeneration. After facial transection, the surviving motor neurons undergo a process of axonal regeneration that starts around 28 day post-injury (dpi) and leads to functional recovery of the facial muscle kynesis. Previous studies performed in our group with transgenic mice strains producing either IL6 or IL10 under the GFAP promoter (GFAP-IL6Tg and GFAP-IL10Tg mice) have shown that these cytokines are able to modulate the CNS response after FNA. Importantly, a decrease of neuronal survival was observed in GFAP-IL6Tg mice respect to wild-type (WT) after 21 dpi, while GFAP-IL10Tg mice showed an increase in motor neuron survival. However, the effect of both cytokines in axonal regeneration has not been explored yet. The objective of this study is to investigate the effects of IL6 and IL10 production in nerve regeneration after FNA. To accomplish this aim, GFAP-IL6Tg and GFAP-IL10Tg adult mice and their respective WT controls underwent FNA. Motor neuron survival was analysed on cryostat sections stained with toluidine blue at 42 dpi. To assess axonal regeneration, 4% solution of Fluoro-Gold (FG), a retrograde fluorescent marker, was injected at 35 dpi in the whiskerpads of both hemispheres, and motor neuron labeling was evaluated at 42 dpi. In addition, the regeneration-associated molecule CD44 was studied from 3 to 28 dpi. Our observations revealed a decrease in the total number of FN motor neurons in the injured side respect to the contralateral side at 42 dpi in all experimental groups. However, no significant differences on neuronal survival were found when comparing the respective experimental groups. In contrast, FG analysis revealed a lower number of regenerating motor neurons targeting the vibrissae in GFAP-IL6Tg mice than in their WT littermates, while GFAP-IL10Tg motor neurons did not show any differences in FG labeling respect to WT. Finally, the study of CD44 showed a decrease at all time-points analyzed in GFAP-IL6Tg mice and an

increase of this molecule in the GFAP-IL10Tg mice at 14 and 28 dpi respect to the WT. In conclusion, our findings indicate that astrocyte-targeted production of either IL6 or IL10 has not a direct impact on long-term neuron survival but may affect the nerve regeneration process after FNA. Further studies are necessary to elucidate the mechanisms of both IL6 and IL10 cytokine production in the dynamics of peripheral axonal regeneration and to assess their influence in target reconnection.

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Poster

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: 7R01AG033679-05

Glenn Award for Research in Biological Mechanisms of Aging

Title: Role of inflammation in impaired autophagy and mitophagy in aging brain

Authors: ***L. L. DUGAN**[1], Q. TANG[2], P. DEMEDIUK[2], J. SASKOWSKI[2];
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Abstract: Autophagy is the choreographed set of processes that eukaryotic cells use to deliver cytoplasmic components to lysosomes for degradation. Autophagy is a highly evolutionarily conserved system activated in response to nutrient deprivation or cellular stress to allow macromolecules to be recycled, and damaged organelles to be degraded and removed. Despite its name (*self-eating*), autophagy is predominantly cytoprotective, and has emerged as a key determinant of lifespan and broad anti-aging effects in organisms ranging from yeast to mammals. Because aging is associated with low-grade inflammatory pathway induction in humans and animal models, we asked whether inflammation might impair autophagy in aging brain. We found large aggregates of p62 deposited throughout hippocampus and cortex by, as well as increased p62 levels on Western blot in aged wild-type mice. p62 specifically targets protein "cargo" to phagosomes for ultimate lysosomal degradation, but is, itself, degraded in lysosomes, thus providing a marker for effective lysosome activity. Accumulation of p62 in brain was observed in the presence of *increased* LC3B II expression by immunofluorescence and Western blot, consistent with inhibition of autophagic flux at the level of lysosomes. In addition,

we observed co-localization of multiple other proteins with p62 aggregates, suggesting a general defect in lysosomal proteolysis. Among these were key mitophagy proteins pink1 and parkin, frequently localized to structures resembling partially degraded mitochondria, suggesting impaired mitophagy as well. Ineffective mitophagy is associated with increased inflammation, and in turn, inflammatory induction of NADPH oxidase (Nox) has been reported to directly impair lysosome activity, so we exposed neuronal cultures to either LPS or interleukin-6 (IL-6), and found that both pro-inflammatory mediators caused increased levels of both p62 and LC3B II, thus reproducing the flux inhibition observed *in vivo*. Inhibition of Nox rescued autophagic flux in pilot experiments, studies which are continuing. If age-related inflammation impairs autophagy by inhibiting lysosomes, these results could link inflammation with Alzheimer's and other neurodegenerative diseases in which protein clearance is impaired.

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Poster

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Ministry of Economy and Competitivity, Government of Spain (PK612884)

Title: Astrocyte-targeted IL10 production modifies the microglial/macrophage proliferation rate and regulates the infiltration of monocyte-derived macrophages

Authors: ***M. RECASENS TORNÉ**[1], K. SHRIVASTAVA[1], B. ALMOLDA[1], I. CAMPBELL[2], B. GONZÁLEZ[1], B. CASTELLANO[1];
[1]Autonomous Univ. of Barcelona, Bellaterra, Spain; [2]Sch. of Mol. Biosci., Sydney, Australia

Abstract: When CNS homeostasis is altered, microglial cells become rapidly activated, proliferate and release a broad range of molecules, including cytokines. Among the plethora of molecules involved in the regulation of microglial activation and monocyte infiltration, cytokines are considered crucial. Although production of interleukin-10 (IL10) by activated astrocytes and microglia has been demonstrated after different types of injuries, the specific role played by IL10 modulating microglial cells remains unclear. Hence, the objective of this study was to evaluate the effects of transgenic astrocyte IL10 production on microglial activation associated with CNS axonal anterograde degeneration. For this purpose, unilateral perforant pathway transection (PPT) was performed in transgenic mice GFAP-IL10Tg and their corresponding wild types (WT) littermates. At 2, 3, 7, 14 and 21 days post-lesion (dpl), animals were intracardiacally perfused

and the hippocampal areas of projection processed for immunohistochemistry (IHC), flow cytometry and ELISA. Our results showed that the non-lesioned GFAP-IL10Tg animals showed an increase in the number of microglial/macrophages (monitored with Pu.1 IHC) compared to WT. After PPT, the cell density was higher in GFAP-IL10Tg than in WT. However, the GFAP-IL10Tg mice showed an important reduction of microglial proliferation (phosphohistone-3 IHC) correlating with a decrease of the expression of macrophage colony -stimulating factor receptor (M-CSFR) and IL5, both molecules are involved in the microglial proliferation. Hence, since the increase of Pu.1+ cells observed in GFAP-IL10Tg was not due to microglial proliferation, we analyse the evolution of microglial/macrophage population after PPT. Flow cytometry revealed that the increase in microglia/macrophage cell density observed in GFAP-IL10Tg was due to a specific increase of $CD11b^{+}/CD45^{high}$ population showing a decrease of MHCII, ICOSL and CD11c expression. Another interesting result is that GFAP-IL10Tg mice showed an increase of $CD11b^{+}/CD45^{high}$/Ly6C cells assuming that the number of infiltrated cells was higher than WT. Correlating with this results, the expression levels of IP10 (chemokine involved in cell recruitment) were higher in GFAP-IL10Tg than WT. No differences were found in microglia/macrophage cell death between Tg and WT animals. In conclusion, this study demonstrated that astrocyte-targeted IL10 production induces 1) an important reduction of microglial proliferation 2) an increase of microglial/macrophages cell density 3) an increases in the number of infiltrated cells.

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Poster

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: The work was supported by NIH Intramural funds.

Title: Astrocyte turnover in a mouse model of LPS-induced brain inflammation

Authors: M. LEE, C. TREXLER, A. NATH, *J. P. STEINER;
NINDS Translational Neurosci. Ctr., Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD

Abstract: The elimination of the latent reservoir can be critical to achieving HIV eradication. Astrocytes are generally considered as long-lived cells with low rates of cell turnover, thus

serving as a long-term reservoir for HIV infection. However, no detailed studies of the degree to which astrocytes proliferate or die have been reported. Here, we determined the turnover rate of astrocytes and determined if the turnover rate can be accelerated in an inflammatory condition. The rate of turnover of cells can be measured in terms of the time between mitosis and death, or between two mitoses. We designed a BrdU pulse-chase experiment to track cell proliferation and death. The experiment was carried out with male C57BL/6 mice about 6 weeks of age when the experiment began. Mice received four injections of BrdU administered three hours apart. Half of the mice received a single injection of LPS 1 h after the first BrdU injection to stimulate brain inflammation. The other half received an injection of PBS as a control. Brain tissues were collected at day 1, 2, 3, 5, 7, 14, 28, 42, 56 and 70 after the first BrdU injection. Four mice were euthanized at each time point. Our data provides strong evidence for moderate astrocyte turnover in the mouse corpus callosum. LPS treatment shortened the astrocyte turnover period. Additionally, the short entry time for GFAP+ BrdU+ cells indicates that mature astrocytes may be capable of replicating, as glial progenitor cells take several weeks to mature into GFAP+ astrocytes after dividing. These results support the idea that an inflammatory response can increase astrocyte turnover, thus providing a new potential mechanism for HIV treatment.

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Poster

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01AA016959

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Title: Isolated microglia express activation markers after binge alcohol exposure in adult rat

Authors: ***H. PENG**, K. NIXON;
Pharmaceut. Sci., Univ. of Kentucky, Lexington, KY

Abstract: Microglia activation and neuroinflammation are common features of neurodegenerative conditions, including alcohol use disorders (AUDs). When activated, microglia span a continuum of diverse phenotypes ranging from classically activated, pro-inflammatory (M1-like) microglia to alternatively activated, growth-promoting microglia/macrophages. Identifying microglia activation states is critical for understanding the

role of microglia in the pathogenesis of AUDs. In this study, male adult rats were gavaged with 25% (w/v) ethanol or isocaloric control diet every 8 hours for 4 days following a modified Majchrowicz protocol, which resulted in an 8.5 ± 1.1 g/kg/d ethanol dose. Rats were sacrificed 2 days after alcohol exposure since previous studies show that indices of microglia activation peak at this time. Microglia were isolated from hippocampus and entorhinal cortex by Percoll density gradient centrifugation. Cells were labeled with microglia surface antigens for activation and analyzed by flow cytometry. Consistent with prior studies, isolated cells yielded a highly enriched population of brain microglia (>95% pure) as evidenced by staining for the microglia antigen CD11b. We compared ethanol's effects on the activation markers (MHC-II, CD86, and CD32) expressed on microglia isolated from the entorhinal cortex and hippocampus of adult rats. Results revealed that ethanol increased the expression of MHC-II, CD86, and CD32 compared to controls. MHC expression increased from 2.5%±0.3 (control) to 19.0%±9.9 (ethanol) in the entorhinal cortex and from 5.3%±2.2 (control) to 21.5%±3.9 (ethanol; $p<0.05$) in the hippocampus. CD32 expression increased from 16.5%±13.2 (control) to 62.3%±4.1 (ethanol) in the entorhinal cortex and from 10.5%±6.3 (control) to 56.8%±3.2 (ethanol; $p<0.05$) in the hippocampus. CD86 expression increased from 2.1%±0.2 (control) to 18.1%±9.5 (ethanol) in the entorhinal cortex, and from 4.3%±1.9 (control) to 20.7%±2.7 (ethanol; $p<0.05$) in the hippocampus. These data support that flow cytometric analysis of isolated, enriched microglia can quantitatively measure microglia polarization state, which complements our published findings in immunohistochemistry and ELISA. Understanding microglia phenotype will be especially critical in pharmacologically reducing or redirecting neuroinflammatory signaling in the treatment of AUDs. (Supported by R01AA016959, R03 NS089433)

Disclosures: H. Peng: None. **K. Nixon:** None.

Poster

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Comparative study of melatonin against the inmunomodulators treatments (interferon beta and glatiramer acetate) in a model of experimental autoimmune encephalitis

Authors: *E. J. RAMOS GONZALEZ[1], O. BITZER QUINTERO[2], L. RAMIREZ JIRANO[2], G. ORTIZ[2];
[1]Neuroimmunomodulation, Ctr. De Investigacion Biomedica De Occidente, Guadalajara, Mexico; [2]Ctr. de Investigacion Biomedica de Occidente, Guadalajara, Mexico

Abstract: INTRODUCTION
Multiple sclerosis (MS) is a chronic autoimmune demyelinating disease of the central nervous system (CNS) leading to neuroinflammation; the animal model of MS is the experimental autoimmune encephalitis (EAE), which is also an autoimmune disease of the CNS. MS classically has been treated with immunomodulators such as interferon beta (IFN-beta) and glatiramer acetate (GA). Melatonin (MLT) is known for its role in many physiological processes, within which highlights the modulation of the immune system, which could be related to the suppression of autoimmune diseases, including EAE.
MS is a chronic inflammatory demyelinating disease of the CNS leading to a cumulative and irreversible damage. To date, there have been no studies comparative of immunomodulatory treatments (IFN - beta and AG) classically prescriben to treat MS and MLT in the animal model (EAE) according to the signs and symptoms observed clinically and expression of proinflammatory cytokines.
OBJECTIVE
Compare the immunomodulatory effect of the administration of melatonin against treatments commonly used in MS (IFN-β and AG) in a murine model of experimental autoimmune encephalitis.
METHODS
Experimental study with male Sprague Dawley adult rats; experimental groups (8): control, EAE, IFN-β, AG, MLT, EAE-IFNß, EAE-AG, EAE-MLT with 16 animals per group; Dosage: IFN-β 8000U every third day, AG 0.5mg/ kg daily and MLT 10mg/ kg daily for 12 days; scale neurological evaluation: grado0 = normal rat, grade 1 = partial loss of tonicity of the tail, grade 2 = loss of tonicity of the tail, grade 3 = unsteady gait and mild paralysis, grade 4 = paralysis of hind limbs, grade 5 = moribund or death. The expression of these proinflammatory cytokine TNF-α, IL-1β and IL-12 was evaluated in cerebrospinal fluid with real time PCR.
RESULTS
Administering INF-β, AG and MLT produces an attenuation of clinical signs and symptoms of EAE to a different scale and at different times for each experimental group.
No expression of proinflammatory cytokines under any of the therapeutic interventions of the experimental groups is observed.
CONCLUSION
Melatonin administration exhibits immunomodulatory effects compared to the treatments used in multiple sclerosis.

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Poster

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: 1P20GM109025-01A1

Title: The effects of insulin impairments in cx3cr1 knockout mice on neuroinflammation

Authors: *A. S. MURTISHAW[1], M. M. BOLTON[2], J. W. KINNEY[2];
[1]Neurosci., UNLV Neurosci. Doctoral Student, Las Vegas, NV; [2]Psychology/Neuroscience, Univ. of Nevada, Las Vegas, Las Vegas, NV

Abstract: Alzheimer's disease (AD) and vascular dementia (VaD) are the two most common forms of dementia. Inflammation and insulin signaling perturbations have emerged as important risk factors associated with both AD and VaD. Data have also demonstrated an interaction between alterations in insulin signaling and inflammation. Many studies support the relationship between AD-VaD and metabolic disorders, particularly diabetes mellitus. Fractalkine (CX3CL1) is a chemokine that has been shown to protect the brain from the damaging consequences of chronic inflammation by interacting with its obligate receptor (CX3CR1) on microglia. Additionally, recent studies have demonstrated that the CX3CL1/CX3CR1 system plays a regulatory role in pancreatic β-cell function and insulin secretion. Our lab has previously demonstrated a low-dose, staggered administration of streptozotocin (STZ) results in hyperglemcia, cognitive impairments, and increased neuroinflammation. To further explore the relationship that fractalkine plays with insulin signaling we administered our low-dose, staggered STZ protocol in CX3CR1 knockout mice to evaluate if the alterations in AD related pathologies and neuroinflammation previously observed are altered in the CX3CR1 KO mice. Animals were run through a standard behavioral and health screen before beginning novel object recognition and cued and contextual fear conditioning. Following completion of behavioral training, brains were removed for biochemical examination. The hippocampus, cortex, and hypothalamus were investigated for markers of inflammation, histopathological alterations relevant to AD, and cerebrovascular abnormalities associated with VaD.

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Poster

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Title: Neonatal exposure to interleukin-1beta enhances adult vulnerability to rotenone neurotoxicity in the nigrostriatal dopaminergic system

Authors: *L.-W. FAN[1], L.-T. TIEN[4], J. W. LEE[1], S. LU[1], R. C. S. LIN[2], X. DAI[3], N. B. OJEDA[1], A. J. BHATT[1], Y. PANG[1];
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Abstract: Early life brain inflammation has been proposed to play important roles in the development of neurodegenerative disorders in adult life. Our previous studies showed that interleukin-1β (IL-1β), a proinflammatory cytokine, plays an important role in mediating dopaminergic neuronal injury in the neonatal rat brain. To examine whether neonatal IL-1β exposure enhances dopaminergic neuron susceptibility to rotenone neurotoxicity at adult ages, Sprague-Dawley male rats at postnatal day 5 (P5) were pre-treated with IL-1β (1 μg/kg) via intracerebral injection, and then challenged with rotenone through subcutaneous mini-pump infusion (1.25 mg/kg per day for 14 days) at P70. A single IL-1β exposure resulted in motor function deficits during the developmental period but were spontaneously recoverable by P70. Single IL-1β exposure also suppressed tyrosine hydroxylase (TH) expression in the substantia nigra (SN) at P70. A low dose of rotenone treatment, which itself does not induce motor deficits in rats, resulted in Parkinsonism-like symptoms including bradykinesia, akinesia and rigidity in rats with neonatal exposure to IL-1β, but not in those without the neonatal IL-1β exposure. Neonatal IL-1β exposure also enhanced adult susceptibility to rotenone-induced loss of dopaminergic neurons as indicated by reduced numbers of TH+ cells in the SN, and axonal impairment as indicated by decreases in the number of Fluoro-Gold (FG)+ nigrostriatal projecting neurons in P98 rats. These results suggest that perinatal neuroinflammation may enhance adult susceptibility to develop neurodegenerative disorders triggered by environmental toxins at an ordinarily non-toxic or sub-toxic dose. Our model may be useful for studying mechanisms involved in the pathogenesis of nonfamilial Parkinson's disease.

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Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 1P20GM109025-01A1

Title: An evaluation of $gaba_b$ receptors in modulating neuroinflammation

Authors: ***M. M. BOLTON**, A. S. MURTISHAW, K. N. CALVIN, J. W. KINNEY; Psychology, Univ. of Nevada Las Vegas Dept. of Psychology, Las Vegas, NV

Abstract: Alzheimer's disease (AD) is characterized by the accumulation of beta-amyloid plaques (Abeta) and neurofibrillary tangles consisting of hyperphosphorylated tau. In addition to these two core pathologies, neuroinflammation has been observed in AD patients and preclinical animal models of AD. Considerable evidence has demonstrated the sustained inflammatory response, in particular chronic microglia activation promotes Abeta production as well as the hyperphosphorylation of tau through the sustained release and increased levels of several proinflammatory cytokines. These data make understanding the mechanisms driving the inflammatory response and treatment of the inflammation an important target in AD research. In addition to aberrant microglia functioning, the loss of a number of aspects of GABAergic signaling, including $GABA_B$ receptors have been reported in clinical AD populations and animal models of AD. As microglia express functional $GABA_B$ receptors and their activation on microglia appear to provide an anti-inflammatory effect, GABA signaling may decrease proinflammatory cytokine production, serving as a vital anti-inflammatory cascade. Therefore, we sought to investigate the role of $GABA_B$ in neuroinflammation related to AD pathogenesis. In two separate, non-transgenic inflammation mouse models we evaluated the behavioral and biochemical effects of a $GABA_B$ agonist baclofen. Following behavioral testing in novel object recognition and cued and contextual fear conditioning, we examined tissues for number of activated microglia, proinflammatory and anti-inflammatory cytokines, and AD related pathological changes. These projects were performed to evaluate behavioral and/or biochemical

modulation of the inflammatory response that may be beneficial. Our data demonstrate that baclofen treatment was able to modify the immune response and alter both behavioral deficits and AD related pathologies.

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Poster

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Title: Nlrp3 mediates systemic and neurologic inflammatory changes resulting from rotenone exposure in mice

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Abstract: Neuroinflammation is thought to contribute to the progression of neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's (PD). The pesticide rotenone, a mitochondrial toxin, has been identified as a risk factor for the development of PD. Synuclein pathology and neuroinflammation have been observed in the brainstem in mice following rotenone exposure but the molecular mechanisms are unclear. Pro-inflammatory "inflammasomes" are multi-protein complexes that react to intracellular stress and initiate inflammation. NLRP3, a key component of the NLRP3 inflammasome complex, and polymorphisms in *NLRP3* are associated with late onset AD and gastro-inflammatory disorder Crohn's disease. Reports of NLRP3 inflammasome activity in AD and evidence that misfolded synuclein can activate NLRP3 in vitro suggest that the NLRP3 inflammasome has an important role in the development and progression of PD. To determine whether Nlrp3 has a role in the development of Parkinsonism or neuroinflammation, wild-type (WT) and *Nlrp3*$^{-/-}$ mice were exposed to intragastric rotenone over a 6 month time course and evaluated using longitudinal behavioral studies and serologic assays. We observed progressive *Nlrp3*-dependent Parkinson's-like motor abnormalities, progressive *Nlrp3*-dependent suppression of the chemokine eotaxin, and significant elevation of Cxcl1 during the time course of rotenone exposure. Post-mortem

multiplex analysis of brain extracts also identified a significant elevation of Cxcl1 in WT mice exposed to rotenone. In vitro analysis identified a robust *Nlrp3*-dependent induction of Cxcl1 in mixed glial cultures. We determined that rotenone-induced Cxcl1 expression was limited to astrocytes in these primary cultures but that induction of astroglia Cxcl1 was enhanced in the presence of microglia after rotenone exposure. While *Nlrp3* expression was detected primarily in CD11b+ microglia the canonical executors of inflammasome activity, IL1B and IL18, were not significantly induced by rotenone. Further experiments will be conducted to determine how microglia enhance astroglia Cxcl1 thereby improving our understanding of how environmental exposure to disease associated toxins could be translated into systemic and neurologic inflammation.

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Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 606.25/Y15

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Alzheimer's Association New Investigator Research Grant NIRG-14-321722 to RLC

POI HL088052 to JTC

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Title: Mild chronic intermittent hypoxia increases oxidative stress and inflammation in brain regions at risk for neurodegeneration

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Abstract: Age is the highest risk factor for the development of neurodegenerative diseases (ND), such as Alzheimer's disease (AD) and Parkinson's disease (PD). As life expectancy increases, the incidence of ND and associated healthcare costs are projected to rise accordingly. Currently, no cure exists for ND and diagnosis occurs at advanced stages, which foreshadows a financial healthcare crisis. Therefore, early identification of patients at risk for ND may provide opportunities for more effective therapies. Since ND is associated with increased oxidative stress (OS) and inflammation, these markers could be used to identify patients at risk for ND. Multiple environmental factors can be an oxidative stressor, and thus exacerbate ND risk. One such

environmental factor that increases OS is sleep apnea (SA). Severe SA has been well established as a common ND comorbidity. However, it is unknown if mild SA induces OS and associated neuroinflammation in areas associated with ND. To model mild SA in rats, chronic intermittent hypoxia was used. Male rats were exposed to six minute chronic intermittent hypoxia (CIH) cycles, during which oxygen levels were rapidly decreased from normal room air oxygen levels of 21% to 10% and held for 90 seconds. Oxygen levels were then rapidly returned to normal room air levels. This protocol repeated for eight hours a day during the light phase for seven days. Plasma and tissue from brain regions associated with ND were collected and tested for levels of OS and inflammation, using Advanced Oxidative Protein Products (AOPP) and multiplex immunoassays, respectively. Regions of interest were hippocampus (HIPP), entorhinal cortex (ETC), substantia nigra (SN), rostral ventrolateral medulla (RVLM), and solitary tract nucleus (NTS). Plasma OS markers and inflammation were elevated in rats exposed to CIH compared to control rats. Dysregulation of inflammatory markers was observed in the RVLM, NTS, and SN. This may contribute to an altered homeostasis, increasing the risk of ND. Increased inflammation was associated with OS in ETC, a brain region associated with early AD, whereas no effects were observed in the HIPP, a brain region associated with later stages of AD. Therefore, mild CIH can contribute to processes involved in early ND pathology by elevating OS and inflammation in critical brain regions.

Disclosures: **B. Snyder:** None. **B. Shell:** None. **J. Cunningham:** None. **R.L. Cunningham:** None.

Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

Location: Halls B-H

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Program#/Poster#: 606.26/Y16

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01NS073848

Title: Microglia progenitors are influenced by physiological stimuli *In vitro* and *In vivo*

Authors: *G. A. GARDEN, M. ALOI, W. SU, R. DODGE, III, T. LE, J. KANG, J. WEINSTEIN;
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Abstract: Microglia are myeloid cells derived from embryonic yolk sac and migrate into the CNS early in embryogenesis. Adult microglia participate in a variety of physiological processes and pathological responses in adult CNS. Two recent studies have demonstrated that

pharmacological or genetic depletion of adult microglia invokes repopulation from a previously unknown Nestin expressing microglia progenitor population. Here we demonstrate that Nestin expressing CD11b^{pos}/Iba-1^{neg} microglia progenitors reside in the normal adult brain and constitute a substantial portion (10-15%) of the total resident myeloid population. Microglia progenitors can be cultured and further differentiated *in vitro* in a manner that recapitulates the molecular signatures observed during embryogenesis. Cultured microglia progenitors exposed to cytokines including interferon gamma, Interleukin-1 beta and tumor necrosis factor beta suppress Nestin expression and increase expression of mRNAs specifically associated with mature microglia. Nestin^{pos}/CD11b^{pos}/Iba-1^{neg} cells were also isolated from the mixed glial cultures employed to generate cultured neonatal microglia, demonstrating that microglia progenitors in neonatal animals are similar to those observed in adults. To determine if inflammatory stimuli influence microglia progenitors *in vivo*, we performed 15 min. unilateral middle cerebral artery occlusion followed by reperfusion (MCAO/R), a stimulus known to induce an inflammatory reaction and ischemic preconditioning without infarction. Microglia progenitor proliferation was significantly induced ipsilateral to 15 minute MCAO/R 72 hours later. We observed an increase in the number of both microglia progenitors and differentiated microglia ipsilateral to MCAO/R at this time point. Microglia (CD11b^{pos}/F480^{pos}/CD45^{int}) isolated from the cortex ipsilateral to MCAO/R also demonstrated gene expression profiles strongly associated with the induction of cellular proliferation. Taken together, these findings demonstrate that microglia progenitors are a distinct population of CNS cell that generate new microglia *in vitro* and can be recruited to participate in an *in vivo* inflammatory response.

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Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Clayton Medical Research Foundation

Title: Cellular expression of L and H prostaglandin D synthases in rat and mouse brain

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Abstract: D- and J-series prostaglandins (PGs) are active in the central mechanisms of sleep, nociception, thermoregulation and inflammation. They represent one arm of the cyclooxygenase-dependent synthetic pathways from PGH_2, catalyzed by two terminal enzymes, lipocalin- and hematopoietic-prostaglandin D synthases (L- and H-PGDS, respectively). These enzymes are structurally and functionally distinct, and there remains uncertainty regarding their cellular localization and regulation in brain. Because prior studies in our laboratory implicated D/J-type PGs in anti-inflammatory interactions between vascular cell types in immune-to-brain signaling, we used immuno- and hybridization histochemical methods to clarify the PGDS distributions in rat and mouse brains. In rat, L-PGDS-immunoreactivity (ir) and mRNA were seen under basal conditions in the leptomeninges and associated with blood vessels throughout the brain. A majority of vascular cells and a minority of meningeal ones co-stained for the macrophage differentiation antigen, ED2, identifying them as perivascular and meningeal macrophages, respectively. Vascular L-PGDS+ cells never co-labeled for the endothelial cell marker, RECA1. Vascular and meningeal labeling for H-PGDS was also extensively colocalized with the ED2 antigen in both meninges and vasculature, and was seen in activated Iba1-ir microglia surrounding a locus of mechanical damage (cannula track). Both enzymes' expression was mildly upregulated in the early hours following an inflammatory challenge (100 µg/kg LPS, i.p.). A similar overall pattern of results was seen in mouse brain, except that we consistently failed to find evidence for vascular expression of H-PGDS mRNA or protein under either basal or stimulated conditions. These findings identify perivascular macrophages as a likely source of D/J prostaglandins in a position to engage in local, anti-inflammatory interactions with cerebrovascular endothelial cells in immune-to-brain signaling.

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Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 606.28/Y18

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: U54NS083924

Title: The anti-inflammatory role of 4R cembranoid

Authors: W. I. VELEZ[1], F. A. MORALES-VIAS[1], M. LEBRÓN-DAVILA[1], V. WASHINGTON[2], P. A. FERCHMIN[1], *V. A. ETEROVIC[1];
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Abstract: The cembranoid (1S, 2E, 4R, 6R, 7E, 11E)-cembra-2,7,11-triene-4,6-diol (4R) is a natural compound found in tobacco leaves that has been shown to significantly decrease the infarct volume in a rodent model of ischemic stroke. There is evidence that this effect is mediated through alpha 7 nicotinic acetylcholine receptors (a7). The purpose of this study was to determine if 4R treatment decreases inflammatory cytokines and if a7 receptors are involved in this effect. This could explain 4R's beneficial role in reducing brain damage after stroke. C57BL/6J wild type and a7 knockout (a7ko) mice received 6mg/kg of 4R dissolved in DMSO subcutaneously 90 min before an intraperitoneal injection of 1mg/kg LPS. Two hours later blood was collected and cytokines measured by flow cytometry. An LPS time-curve performed in wild type mice showed that TNFalpha peaked at 1 hr, while IL-6, IL-10, and MCP-1 peaked at 2 hrs. LPS treatment significantly increased the levels of all 4 cytokines in both wt and a7ko mice compared to mice receiving saline. Wt mice receiving either vehicle + LPS or 4R + LPS had significantly lower TNFalpha, MCP-1, and IL-10 cytokine levels compared to those receiving LPS alone. However, in the a7ko mice only the vehicle + LPS group had significantly lower levels of IL-6, TNF, MCP-1, and IL-10 compared to the LPS group. Cytokine levels in the 4R + LPS group were not significantly different from those in the LPS group in these mice. T-test analyses showed significant differences between wt and a7ko mice with regard to IL-6 (3,605 ± 1340 versus 10,021 ± 5,100, respectively) and Il-10 (62 ± 36 versus 863 ± 200, respectively) cytokine levels. These data show that cembranoid 4R lowers LPS-induced TNF, MCP-1 and IL-10 levels by an a7-dependent mechanism.

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Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 606.29/Z1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CONACyT

PAEP; PCB, UNAM

Title: Evaluation of the effect of monocyte locomotion inhibitor factor in motor recovery and acute phase gene expression in rats with traumatic spinal cord injury

Authors: *J. S. HERRERA GARCÍA[1,2], A. IBARRA ARIAS[3,4], L. BLANCAS ESPINOZA[1], E. GARCÍA VENCES[3], A. FLORES ROMERO[4], R. SILVA GARCÍA[1];
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Abstract: After spinal cord injury (SCI) occurs, self-destructive mechanisms that cause immediate and chronic neurodegenerative process are triggered; they could harm motor, autonomic, and sensitivity functions. Several works have studied the effects of this inflammatory response and state that the balance between benefic and damaging effects depends in its intensity, in addition, mayor secondary damages occur during the acute phase. There's no fully restorative therapy for SCI, but strategies for modulating inflammation provided evidence that rehabilitation after SCI may be possible. Monocyte locomotion inhibitor factor (MLIF) is a pentapeptide that regulates inflammatory molecules expression and increases the expression of proliferation, angiogenesis, vasculogenesis, and axonal guidance genes *in vitro*. In this context, it is necessary to know the effect of the MLIF on the expression in genes related to neuroprotection, degeneration, and inflammation, in an *in vivo* model. The aim of this study was to evaluate whether MLIF exerts a neuroprotective effect that promotes motor recovery by modulating the acute phase gene expression in rats subjected to SCI. Two experimental designs using 60 Sprague Dawley female rats divided in 5 groups: Sham; PBS; 1, 3 and 7 MLIF applications. All groups underwent a laminectomy at T9 level and a controlled moderated lession was followed by the application of MLIF or PBS as corresponding. The first application of MLIF (200 μg) or PBS were administered in the injury site and subsequent MLIF applications intraperitoneally every 24 hrs. In the first experimental design, the motor ability of the rats treated was tested during 8 weeks using the "BBB" scale. MLIF showed significant differences relative in comparison to the control group (α=0.01). Groups with multiple doses showed better motor recuperations than the group with only one dose. In the second experimental design, 3hrs after the last treatment in each group rats were sacrificed and 1 cm of spinal cord from the injury site was obtained; RNA was purified and microarray analysis expression studies were performed. The modification of expression of different gene families was observed. In qPCR, it was found that MLIF modulates DIVA, Pax6, and XIAP anti-apoptotic genes selectively in the three treatments; while, Casp3 had a lower expression and iNOS had a higher expression, however, it can be argued that when correlating the long-term behavior of individuals, the anti-apoptotic stimulus significantly promotes neuronal survival, thus improving the motor recuperation in rats subjected to a SCI model.

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Poster

606. Mechanisms and Consequences of Peripheral or Central Innate Immune Activation

Location: Halls B-H

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Foundation for Anesthesia Education and Research (FAER) grant A123320 (JZP)

NIH grant NS038079 (MSB & JCB)

Title: Dexmedetomidine modulates immune responses and improves recovery after rat spinal cord injury (SCI)

Authors: *J. Z. PAN[1,3], Y.-W. CHANG[3], A. LIN[2,3], S. LEE[2,3], J. SACRAMENTO[2,3], J. GAO[1,3], Z. SUN[1,3], J. BRESNAHAN[2,3], M. MAZE[1], M. BEATTIE[2,3];
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Abstract: Background
Catecholamines, that are released early after acute spinal cord injury (SCI) as part of the sympathetic nervous system response to injury, stimulate splenic adrenergic receptors and promote monocyte migration into the injury site. Splenectomy immediately prior to SCI leads to reduced monocytes at the injury epicenter, decreased lesion size and better behavioral outcome (Blomster et al., 2013). Attenuation of the sympathetic response improves outcome in experimental stroke, another model of acute neurologic injury (Ajmo et al., 2009). Dexmedetomidine (DEX), a sedative that is commonly used for critically ill patients, including those with spinal trauma, is a sympatholytic agent by virtue of its highly selective alpha-2 adrenergic agonist activity. We hypothesize that DEX can improve functional outcome after SCI by modulating the neuro-immune response.
Methods
Long-Evans female rats received i.p. injection of DEX (25 μg/kg) following unilateral C5 contusion injury (75 kdyne) or C5 laminectomy only. DEX was given at 0, 2, 4, 6h following SCI on the first day and daily single dose on 2d, 3d, 4d and 5d thereafter. Animals were sacrificed at 6h, 24h or 7d after injury. Grooming score and forepaw placement tests were performed on 2d and 7d after SCI to evaluate behavioral recovery. In all groups, spinal cord, serum, and spleen tissues were harvested and analyzed for inflammatory markers. Flow cytometric analysis of peripheral leukocytes was also performed.
Results
Inflammatory markers were assessed in local injured spinal cord tissue at 6h, 1d and 7d post SCI. Compared to injury control, IL-6 was significantly reduced at all three time points, while TLR-4 is down-regulated at 6h after DEX treatment. As a marker for macrophage and microglia, CD11b

expression is decreased in DEX treated group. Further, DEX enhances the expression of Arginase-1 at both 6h and 1d, which suggests its ability to promote an anti-inflammatory response and tissue repair. In a time course study of blood monocyte using flow cytometry, percentage of peripheral CD11b/c positive cells are quickly elevated at 6h, peaked 1d after injury and slowly approach baseline over weeks. At 6h post SCI, these cells are significantly reduced by DEX. However, DEX seems to have no effect on these cells at later time points (1 and 7 days). In behavioral tests, there is a trend of improvement in grooming score and weight support by ipsilateral forelimb in the DEX treated animals.
Conclusions
As a highly selective α2-adrenergic agonist and sympatholytic agent, dexmedetomidine seems to improve functional recovery after acute SCI and modulates both local and systemic immune responses.

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Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 607.01/Z3

Topic: C.08.Stroke

Title: Neovascularization by mechanical barrier disruption procedure infusing systemic erythropoietin in mild ischemic rat model

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Abstract: BACKGROUND: Stroke is one of the most disabled diseases and neovascularization is a promising strategy for acute ischemic stroke. To achieve a successful backward neovascularization from the external cranium with enriched environment, we simply combined mechanical barrier disruption (MBD) on the skull with systemic erythropoietin infusion under the brain ischemic condition. Therefore, this combination is hypothesized by two beneficial modes: (1) MBD is for a new vessel detour from the outside, (2) systemic erythropoietin (EPO) is a boosting drug for vessel maturation without regression. METHODS: Mild ischemic animal model was established by subjecting male Sprague-Dawley rats (250-270g) to bilateral internal carotid artery ligation (bICAL). They were intraperitoneally injected with recombinant human EPO (5000 U/kg) or saline for 3 days and were performed as a 2.5±0.5 mm burr-hole operation on the right side. MBD groups with or without EPO were compared in histology, gene

expressions using quantitative real-time polymerase chain reaction, and postmortem angiography using the india ink stain at 3 phases: 4 (early, T1), 7 and 14 (intermediate, T2), 28 and 84 days (late, T3). RESULTS: Compared to contralateral side without MBD (left), ipsilateral side with MBD showed a successful neovascularization after 14 days (T2): Extra- and intracranial anastomoses were shown in a postmortem angiography at 14 days and vessel density (RECA-1^{+}) was gradually increased since T2. Compared to contralateral MBD side during T1, ipsilateral MBD side showed an increase of pro-angiogenic (angiopoietin 2), inflammatory factors for vessel permeability, and activated M1 type microglia (Iba-1+/CD16/32^{+}). However, there was a suppression of inflammatory responses (those with EPO) in T1. Compared to MBD without EPO group during T3, MBD with EPO group showed the increases of functional and structural vessel maturation (RECA-1^{+}/SMA^{+}/$NG2^{+}$) along with upregulating relevant genes and activated M2 type-microglia (Iba-1^{+}/$CD206^{+}$). Minocycline (45mg/kg) infusion for consecutive 4 days (MIC, inflammatory inhibitor during T1) showed a decreased expression of inflammatory factors and a decreased angiogenesis at T3, even in MBD with EPO group. CONCLUSIONS: Our data suggest that EPO contribute to a long-term stability for angiogenesis representing pro-angiogenic and anti-inflammatory properties, especially under the MBD status. This new method augmenting neovascularization without its regression can reflect a novel therapeutic strategy for ischemic stroke victims.

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Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

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Program#/Poster#: 607.02/Z4

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant NS088608

Title: Differential effects of rapamycin treatment on recurrent synaptic excitation of hilar inhibitory interneurons after focal brain injury in mice

Authors: *C. R. BUTLER[1], J. A. BOYCHUK[1,2,3], B. N. SMITH[1,2,4];
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Abstract: Traumatic brain injury (TBI) is among the most common causes of acquired temporal lobe epilepsy (TLE). Multiple forms of synaptic reorganization occur in the dentate gyrus after

brain injury, which may contribute to TLE development. A murine model of TBI using controlled cortical impact (CCI) injury was used to examine the effect of daily rapamycin treatment (3 mg/kg) on excitatory synaptic reorganization of dentate granule cells (DGCs) and CA3 pyramidal neurons after CCI injury. Increased doublecortin immunolabeling was observed in the ipsilateral DGC layer 2 weeks after CCI injury and mossy fiber sprouting was evident 8-13 weeks post-injury in the ipsilateral hemisphere. Daily rapamycin treatment reduced both the increased doublecortin immunolabeling and mossy fiber sprouting in the ipsilateral dentate gyrus. Using mice that express GFP in a subset of inhibitory neurons (FVB-Tg(GadGFP)4570Swn/J; i.e., GIN mice), whole-cell patch-clamp and on-cell recordings in vitro detected an increase in spontaneous EPSC frequency and action potential firing rate of surviving GFP+ hilar interneurons ipsilateral to CCI injury, relative to cells contralateral to the injury. Relative to CCI injury alone, daily rapamycin treatment for 8-12 weeks after CCI injury resulted in a reduction in the increase in sEPSC frequency and spontaneous firing rate of GFP+ hilar interneurons, but these measures were not normalized to control levels. After CCI injury, GFP+ hilar interneurons ipsilateral to injury exhibited increased evoked responses to glutamate photostimulation of both DGCs and CA3 pyramidal neurons, relative to contralateral and sham controls. Rapamycin treatment reduced responses of ipsilateral GFP+ hilar interneurons to glutamate photostimulation of DGCs, but did not reduce the increased response to glutamate photostimulation of CA3 pyramidal neurons. Rapamycin treatment therefore suppresses synaptic reorganization of DGCs, but not CA3 pyramidal cells after CCI injury. Ongoing experiments using glutamate photolysis will test the hypothesis that rapamycin treatment suppresses excitatory axon sprouting of DGCs, but not other neurons ipsilateral to CCI injury.

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Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

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Program#/Poster#: 607.03/Z5

Topic: C.09. Brain Injury and Trauma

Support: Center of Biomedical Research Excellence Pilot Award(CoBRE P30GM103400/

Title: Enduring erythropoietin repair of cerebral microstructure and diffusion abnormalities following severe infant traumatic brain injury in rats

Authors: *L. L. JANTZIE[1], S. ROBINSON[2];
[1]Neurol., Univ. of New Mexico Dept. of Pediatrics, Albuquerque, NM; [2]Pediatric Neurosurg., Johns Hopkins Univ., Baltimore, MD

Abstract: Traumatic brain injury (TBI) is a leading cause of death and severe morbidity for infants who are born healthy. Despite the high prevalence and incidence of lifelong deficits from TBI suffered by infants, no targeted treatment currently exists to actively promote repair. Using a translatable preclinical model of infant TBI, we hypothesized that diffusion tensor (DT) magnetic resonance imaging (MRI) could be used to quantify outcomes and potential efficacy of the neuro-reparative agent erythropoietin (EPO). To this end, controlled cortical impact (CCI) was performed on postnatal day 12 (P12) rats of both sexes. Anesthetized rats underwent left parietal craniectomy and 0.6mm impact. On post-injury day (pid) 1, injured rats were randomized to 6 doses of intraperitoneal erythropoietin (EPO) 3000U/kg/dose or vehicle (sterile saline) over pid 1-8. Ex vivo MRI was performed on a 4.7T MRI scanner at P15, P45 and P90, and quantified by observers blinded to injury and treatment status. DTI investigation reveals bilateral widespread injury and significant abnormalities of functional anisotropy (FA), axial (AD) and radial diffusivity (RD) developing acutely from P15 through P45 in multiple gray and white matter structures following CCI. Specifically, FA is decreased, and AD and RD increased, in the corpus callosum, and hippocampus and thalamus ipisilateral to CCI compared to sham (n=6-8/group, $p<0.05$ for all). Notably, treatment with EPO reversed changes in AD and RD throughout the white matter, overlying cortex and AD in thalamus both ipsilateral and contralateral to CCI (two-way ANOVA with post-hoc correction, $p=0.011$ and $p=0.029$, respectively). Impaired diffusivity in white and gray matter was sustained at P90, with increases in AD and RD concomitant with reduced structural coherence in the corpus callosum, lateral white matter and cortex. Notably, EPO treatment provided sustained repair of AD and RD microstructural abnormalities bilaterally (i.e. corpus callosum RD: CCI-veh $4.0\pm0.29x10^{-4}$ vs. CCI-EPO $3.4\pm0.02x10^{-4}$ mm^2/s, $p=0.03$). Together, these data indicate that EPO, administered in an extended post-injury dosing paradigm congruent with its mechanisms of action, proved efficacious in reversing microstructural and sustained diffusion abnormalities in developing rats. These data support the use of age-appropriate preclinical models with human clinical trial-compatible imaging biomarkers and outcome measures.

Disclosures: L.L. Jantzie: None. **S. Robinson:** None.

Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

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Topic: C.09. Brain Injury and Trauma

Support: Center of Biomedical Research Excellence Pilot Award(CoBRE P30GM103400/

Title: Erythropoietin reverses cognitive deficits in a model of infant traumatic brain injury

Authors: *A. OPPONG[1], L. L. JANTZIE[2], S. ROBINSON[1];
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Abstract: Traumatic brain injury (TBI) is a leading cause of death and severe morbidity for infants who are born healthy. No targeted treatment currently exists to actively promote repair after early TBI, despite the high incidence of chronic, debilitating deficits. Using a translatable preclinical model of infant TBI, we hypothesized that touchscreen cognitive testing could be used to quantify outcomes and potential efficacy of the neuro-reparative agent erythropoietin (EPO). Controlled cortical impact (CCI) was performed on postnatal day 12 (P12) rats of both sexes. Anesthetized rats underwent left parietal craniectomy and 0.6mm impact. On post-injury day (pid) 1, injured rats were randomized to 6 doses of intraperitoneal erythropoietin (EPO) 3000U/kg/dose or vehicle (sterile saline) over pid 1-8. Similar to the Cambridge Neuropsychological Test Automated Battery (CANTAB) in humans, a touchscreen platform was applied to assess multiple cognitive domains. Beginning at P35, rats were trained on a touchscreen operant platform by blinded investigators. Following successful training, rats performed visual discrimination (VD) and reversal tasks to assess executive function and cognitive flexibility (n=6-8/group, two way ANOVA with Bonferroni correction). Results indicate that Sham, CCI and CCI-EPO rats are able to successfully perform VD with each group requiring a similar number of sessions to passing criteria and committing similar numbers of discrimination errors. However, when assessing reversal learning, only 57.1% of CCI animals successfully passed criteria compared to 100% of Sham and 83.3% of CCI animals treated with EPO. Notably, CCI animals committed significantly more errors and required more correction trials (1114±132) compared to Sham (730±95) and CCI-EPO (652±61, $p<0.05$ for all), concomitant with increased perseveration (all $p<0.05$). Reaction and magazine latencies were similar for each group and did not differ between VD and reversal platforms. Taken together, EPO treatment ameliorates deficits on a touchscreen platform, including error reduction and perseveration, and restores cognitive flexibility. To our knowledge, this is the first demonstration using the highly-translatable, sophisticated cognitive testing of the touchscreen platform for TBI. EPO, administered in an extended post-injury dosing paradigm congruent with its mechanisms of action, proved efficacious in reversing functional impairment after TBI in developing rats.

Disclosures: A. Oppong: None. **L.L. Jantzie:** None. **S. Robinson:** None.

Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 607.05/Z7

Topic: C.08.Stroke

Support: 3i TEKES

Title: Effects of intraventricular cdnf, manf and gdnf injections on neuroblast proliferation and migration after cortical stroke in adult rats

Authors: *K.-Y. TSENG[1], J. ANTTILA[1], K. MATLIK[1], A. DOMANSKYI[1], M. SAARMA[1,2], R. TUOMINEN[2], M. AIRAVAARA[1];
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Abstract: Objective: Mesencephalic astrocyte-derived neurotrophic factor (MANF) and cerebral dopamine neurotrophic factor form a novel evolutionally conserved family of neurotrophic factors. Previous studies have shown that glial cell line-derived neurotrophic factor (GDNF) and MANF pretreatment reduce ischemic brain injury and promote behavior recovery, and that GDNF induces neurogenesis after striatal stroke. The purpose of this study was to study the effects of MANF, CDNF and GDNF treatments on neurogenesis in stroke rats. **Material and Methods:** Adult rats were subjective to 90 minutes middle cerebral artery occlusion (MCAO) and divided into four groups based on their neurological scores. Recombinant human MANF, CDNF, GDNF proteins (10 µg) or phosphate buffered saline (PBS) was administrated to rats via intraventricular injections on days 3, 7, 10 post-stroke. Proliferating cells were labeled with 5´-bromo-2´deoxyuridine (BrdU) injections from day 4 to day 10. Fourteen days after stroke rats were perfused and brains collected for immunohistochemistry. **Result:** Stroke increased the number of doublecortin+ cells in the ipsilateral SVZ post-stroke. GDNF increased BrdU+ cells in ipsilateral SVZ and the number of doublecortin+ cells in the striatum. MANF did not have effect on number of BrdU+ cells in the SVZ but it increased number of doublecortin+ in the corpus callosum and the peri-infarction zone. We did not observe effects with CDNF. **Conclusion:** Taken together, these data suggest that intraventricular injections of MANF after post-stroke promoted the migration of endogenous neuroblasts from the SVZ to the lesioned cortex in adult rats of stroke.

Disclosures: K. Tseng: None. **J. Anttila:** None. **K. matlik:** None. **A. Domanskyi:** None. **M. Saarma:** None. **R. Tuominen:** None. **M. Airavaara:** None.

Poster

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Program#/Poster#: 607.06/Z8

Topic: C.08.Stroke

Support: CIHR

NSERC

OGS

Heart and Stroke Foundation

Title: Promoting post-stroke plasticity with local delivery of brain-derived neurotrophic factor

Authors: *J. M. OBERMEYER[1], A. TULADHAR[2], S. L. PAYNE[1], C. M. MORSHEAD[3], M. S. SHOICHET[1,2,4];
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Abstract: Stroke is a devastating neurological event, affecting approximately 5 million people each year. As there is currently no treatment that will regenerate damaged brain tissue, stroke survivors suffer from permanent disabilities and memory loss that greatly impact quality of life. Administration of a known agent of plasticity, Brain-Derived Neurotrophic Factor (BDNF), has the potential to facilitate the rearrangement necessary to compensate for stroke-induced deficiencies; however, delivery of this protein to the brain remains a challenge[1]. We have previously developed a biocomposite composed of a hyaluronan and methyl cellulose hydrogel (HAMC) and poly(lactic-co-glycolic acid) (PLGA) nanoparticles in order to circumvent the blood brain barrier and provide local, sustained release of therapeutics[2]. In this work, we optimize this biocomposite for the delivery of BDNF and evaluate efficacy of this delivery strategy in a rat model of stroke injury.
BDNF was encapsulated in PLGA nanoparticles using a double emulsion technique. These nanoparticles were mixed with hyaluronan and methyl cellulose to create the PLGA-HAMC biocomposite and the release of BDNF from this system was studied *in vitro*. To measure the extent of penetration of locally delivered BDNF into stroke-injured brain tissue, endothelin-1 was used to create stroke lesions in male Sprague-Dawley rats and the biocomposite was injected onto the surface of the cortex above the lesion. Animals were sacrificed at 13 and 20 days post-injury and enzyme-linked immunosorbant assay (ELISA) was used to measure BDNF concentration in the tissue.
In vitro, the release of BDNF from the HAMC hydrogel alone reached completion by three days,

whereas BDNF release from the PLGA-HAMC system was sustained out to 28 days with no initial burst release. A dorsal root ganglion bioassay revealed that the BDNF released from this system was still bioactive at each sample time point. *In vivo*, BDNF diffused from the biocomposite on the surface of the cortex into the tissue and was detectable above endogenous levels at all depths tested.
The PLGA-HAMC delivery system achieved sustained, local release of bioactive BDNF to the stroke-injured rat brain. Ongoing studies will evaluate the effect of locally
delivered BDNF on synaptic plasticity and functional recovery in the endothelin-1 rat model of stroke.
References:
1.
Cowansage, K. K., LeDoux, J. E. & Monfils, M.-H. *Curr. Mol. Pharmacol.* **3,** 12-29 (2010).
2.
Cooke, M. J., Wang, Y., Morshead, C. M. & Shoichet, M. S. *Biomaterials* **32,** 5688-97 (2011).

Disclosures: J.M. Obermeyer: None. **A. Tuladhar:** None. **S.L. Payne:** None. **C.M. Morshead:** None. **M.S. Shoichet:** None.

Poster

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Program#/Poster#: 607.07/Z9

Topic: C.09. Brain Injury and Trauma

Support: Roskamp Foundation

Title: Dihydropiridine calcium channel antagonist (Nilvadipine) is potential drug against repetitive mild TBI

Authors: *A. MORIN, B. MOUZON, S. FERGUSON, F. CRAWFORD;
Roskamp Inst., Sarasota, FL

Abstract: Traumatic brain injury (TBI) is the recognized cause of disability in various population groups including military/veterans and athletes, and has long been known as a risk factor for neurodegenerative disorders such as Alzheimer's disease (AD). Currently, there is no efficient treatment for TBI due to complex and diverse neuropathological processes occurring after injury. However, we propose that certain drugs which are used to ameliorate AD conditions may also show positive changes in TBI. For this purpose, we are testing nilvadipine, calcium channel blocker (CCB), in a mouse model of repetitive mild TBI (r-mTBI). Nilvadipine is known

as an anti-hypertensive drug, but was also shown in our laboratory as AD preventive drug via reduction of Aβ accumulation, and is now under clinical trial, Phase III in Europe. To verify nilvadipine effects after r-mTBI, we are using aged htau mice (expressing all six isoforms of human tau) which show age-related tau pathological features. Animals subject to five hits with an inter-injury interval of 48hrs or r-sham in order to control for the effects of repeated anesthesia followed intraperitoneal (IP) injections of nilvadipine during 21 days. A battery of behavioral tests is currently ongoing to assess cognitive impairments. Rotarod showed positive performance of motor function in nilvadipine treated mice compared to vehicle only group. Barnes Maze and optomotor response were used to assess spatial memory and vision. Moreover, we are planning to collect brain samples 1 week after final injection for immunohistochemistry (IHC) analysis and to look at inflammation level using Iba1, GFAP and APP as biomarkers for activated microglia, astrogliosis and axonal damage, respectively.

Disclosures: **A. Morin:** None. **B. Mouzon:** None. **S. Ferguson:** None. **F. Crawford:** None.

Poster

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Topic: C.08.Stroke

Support: Dr John P and Therese E Mulcahy Endowed Professorship in Ophthalmology (SK)

Department of Veterans Affais (GLK, SK)

Title: The aminopeptidase P2 inhibitor, ST-115, improves functional outcomes after experimental stroke.

Authors: *S. KAJA[1,3,2], S.-Y. TSAI[3], A. ROCKWELL[1], E. SAVARESE[1], V. RAO[2], G. L. KARTJE[3,2], W. H. SIMMONS[2];
[1]Ophthalmology, [2]Mol. Pharmacol. & Therapeut., Loyola Univ. Chicago, Maywood, IL; [3]Res. Service, Edward Hines Jr VA Hosp., Hines, IL

Abstract: Stroke remains the fifth leading cause of death and the leading cause of disability in the US. Previous studies have shown that reperfusion injury is positively associated with the extent of stroke damage. There is an urgent clinical need for novel safe and efficacious therapies that prevent the deleterious effects of reperfusion injury. Apstatin is a selective aminopeptidase P2 blocker that has previously been shown to be highly effective in preventing cardiac reperfusion injury. Aminopeptidase P2 is localized at the luminal plasma membrane in

endothelial cells, where it inactivates the peptide hormone bradykinin through hydrolysis. Here we tested the hypothesis whether the apstatin analog, ST-115, can prevent ischemia/reperfusion injury and improve functional outcomes and retinal degeneration in an experimental model for stroke. Male Sprague-Dawley rats (2 months of age) were subjected to experimental stroke surgery by transient (45 min) bilateral occlusion of the common carotid artery and unilateral occlusion of the middle cerebral artery, followed by either 48 hr or 2 wk of survival time. Animals received either saline, or the apstatin analog ST-115 (16 μg/kg), which is 1000-times more potent than apstatin (IC_{50} = 3.7 nM) by intravenous injection immediately prior to initiation of reperfusion. At 48 hrs, ST-115-treated animals showed a highly statistically significant improvement of acute neurological deficits as assessed by the NeuroScore paradigm, which tests abnormal forelimb posture, rearing, and weight bearing (2.3 ± 0.2 and 0.2 ± 0.2; $P<0.01$), and a statistically significant reduction in the volume of ischemia-induced lesions as quantified by TTC staining. In order to test long-term functional outcomes, we assessed the skilled forelimb reaching task in another cohort after 2 wk of survival. ST-115-treated animals performed significantly better than saline-treated controls ($P<0.001$) and showed no statistically significant difference in the reaching score from baseline ($P=0.46$). We further characterized retinal damage, which is clinically associated with stroke and ischemia/reperfusion injury. ST-115 reduced the number of apoptotic retinal ganglion cells (RGCs) from 62.1% to 40.5% ($P<0.01$), as assessed by fluorescent TUNEL staining on frozen sections. Similarly, ST-115 improved retinal thinning and reduced expression of astrocytic and microglial markers, glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (Iba1). Our data provide strong proof-of-concept and feasibility data supporting the development of apstatin analogs for targeted therapy against ischemia/reperfusion injury after stroke.

Disclosures: **S. Kaja:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); K&P Scientific LLC, Experimentica Ltd.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); K&P Scientific LLC, Experimentica Ltd.. F. Consulting Fees (e.g., advisory boards); Experimentica Ltd.. **S. Tsai:** None. **A. Rockwell:** None. **E. Savarese:** None. **V. Rao:** None. **G.L. Kartje:** None. **W.H. Simmons:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Patent US9212206 B1.

Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

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Topic: C.09. Brain Injury and Trauma

Support: NIH 5R21NS087077-02

NIH 5R01NS052325-07

Title: A role for neuropeptide signaling in regulating *C. elegans* response to anoxia

Authors: *S. DOSHI[1], R. G. KALB[1,2];
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Abstract: While the brain is 2% of the body's mass, it utilizes 20% of all consumed oxygen to maintain its energy needs. As a result, the nervous system is very sensitive to oxygen availability, and neurons can alter their metabolic state and activity upon detecting a decrease in oxygen. Neuronal injury caused by reduced oxygen (hypoxia or anoxia) has severe implications in development and adulthood, leading to impaired brain function and even death. In this study, we use the nematode *C. elegans* to understand the molecular regulation of anoxic injury. The worm responds to hypoxia and anoxia using distinct molecular pathways, and while the genetic regulation of the hypoxic response has been well characterized in the worm, less is known about the response to anoxic environments. Here, we find a novel role for neuropeptide signaling in regulating a specific response to anoxia using genetic manipulations afforded by this model organism. Loss of function mutants in neuropeptide maturation (egl-3) as well as neuropeptide release (unc-31) have a significant survival advantage after 48hr anoxia compared to wild-type N2 animals. Tissue-specific loss of egl-3 demonstrates that the nervous system is necessary for this effect, confirming that neuropeptides, and not other peptidergic hormones, mediate this regulatory response. The role of neuropeptide signaling is also specific to anoxic stress as it does not protect against other stressors such as heat or ER stress. This response is independent of many canonical stress resistance factors (daf-16, hsf-1, skn-1). It is also independent other known genes involved in hypoxia and anoxia, such as hif-1 and nsy-1. Others have reported that the homolog of yeast longevity assurance gene 2 (hyl-2), a ceramide synthase, protects *C. elegans* from anoxia as hyl-2 mutants are very poor survivors of anoxic stress. We made double mutants to test whether loss of neuropeptide secretion is dependent on hyl-2 for its protective effect. Our results indicate that hyl-2 is indeed necessary for the protective effect conferred by loss of neuropeptide signaling. Ongoing work aims to describe the mechanistic link between unc-31/egl-3 and hyl-2 in regulating this response. We are also working to define the specific neuropeptide(s) and the cell-types responsible for neuropeptide-mediated death under anoxia. This work sheds light on a novel and specific pathway regulating the response to severe oxygen deprivation, and could have implications for a variety of human conditions including, among others, hypoxic ischemic encephalopathy, stroke and injury to the brain.

Disclosures: S. Doshi: None. **R.G. Kalb:** None.

Poster

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Topic: C.09. Brain Injury and Trauma

Support: DARPA Grant HR0011-13-2-0017

NMSS Grant RG-4666-A-2

Title: Targeted delivery of imaging and therapeutic compounds into acute brain injuries

Authors: A. P. MANN[1], P. SCODELLER[1], S. HUSSAIN[1], J. JOO[2], E. KWON[3], G. B. BRAUN[1], T. MOLDER[4], Z.-G. SHE[1], *B. RANSCHT[1], S. KRAJEWSKI[1], T. TEESALU[4], S. BHATIA[3], M. SAILOR[2], E. RUOSLAHTI[1];
[1]Sanford Burnham Prebys Med. Discovery Inst., LA Jolla, CA; [2]Univ. of California San Diego, San Diego, CA; [3]MIT, Boston, MA; [4]Inst. of Biomedicine and Translational Med., Univ. of Tartu, Tartu, Estonia

Abstract: Traumatic brain injury (TBI) is the leading cause of death and disability in the most active segment of the population. At present, no pharmacological agent is approved for the treatment of TBI. The enormous human suffering and financial burden of TBI provides a compelling rationale for the development of novel agents and approaches to TBI management. Using *in vivo* phage screening in an animal model of TBI, we have identified a novel brain injury targeting peptide (BIP) that, when systemically injected, specifically homes to sites of injury in penetrating and non-penetrating (controlled cortical impact) brain injury models. The target epitope for BIP peptide appears to be within a proteoglycan complex that is upregulated in mouse and human brain injuries. We have used BIP to enhance the accumulation of systemically administered molecules ranging from a drug-sized molecule to nanoparticles to sites of brain injury. BIP-coated nanoparticles containing silencing oligonucleotides provided first evidence of gene silencing in injured brain parenchyma by systemically administered siRNA. Importantly, BIP also showed selective binding to human brain injury lesions, which supports the potential application of this delivery strategy in humans. The effect of targeted delivery of therapeutics using BIP on brain injury is currently being investigated. Together, these findings present a readily accessible and effective targeting strategy for the delivery of therapeutics in clinical management of acute brain injuries.

Disclosures: **A.P. Mann:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AivoCode. **P. Scodeller:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder. **S. Hussain:** A.

Employment/Salary (full or part-time): AivoCode. 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; AivoCode. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AivoCode. **J. Joo:** None. **E. Kwon:** None. **G.B. Braun:** None. **T. Molder:** None. **Z. She:** None. **B. Ranscht:** None. **S. Krajewski:** None. **T. Teesalu:** None. **S. Bhatia:** None. **M. Sailor:** None. **E. Ruoslahti:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AivoCode.

Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

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Topic: C.09. Brain Injury and Trauma

Support: 1RO1NS091218

Title: cPLA2 activation leads to lysosomal damage and autophagy impairment after TBI

Authors: *C. SARKAR[1], S. LIU[2], N. HEGDEKAR[1], J. PETER[1], A. I. FADEN[1], M. M. LIPINSKI[1];
[1]Shock, Trauma and Anesthesiol. Res. (STAR) Center, Dept. of Anesthesiol., [2]Dept. of Orthopaedics, Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Autophagy, a lysosome dependent major cellular degradative process, plays important role in maintaining cellular homeostasis particularly in cells like neurons. Autophagic dysregulation has been implicated as one of the major causes of neuronal cell death in several neurodegenerative diseases. Recently we also have demonstrated impairment of autophagy following controlled cortical impact (CCI) induced traumatic brain injury (TBI) in mice. We observed block of autophagosome degradation mainly within neuron at early time point after TBI. Our data indicated that it is at least in part due to lysosomal dysfunction, as evidenced by lower protein levels and enzymatic activity of the lysosomal enzyme, cathepsin D (CTSD) in injured cortex at day 1 after TBI. In the current study we further explored the mechanism of lysosomal defect following TBI. We observed leakage of lysosomal content into the cytosol due to lysosomal membrane damage after brain injury. This correlated with the activation of cytosolic phospholipase A2 (cPLA2), an enzyme that cleaves fatty acyl linkage in the phospholipid of cellular membranes. We observed enhanced phosphorylation of cPLA2 within mouse cortex soon after injury. Furthermore, elevated level of cPLA2 was also observed within

neurons where autophagosomes accumulate following brain injury. Consistently with the possibility that cPLA2 activity may damage lysosomal membranes and inhibit autophagy, block of autophagosome degradation was detected *in vitro* in H4 cells and rat cortical neurons treated with cPLA2 activator ceramide-1-phosphate (C1P). This was due to the lysosomal damage caused by cPLA2 activation as evidenced by lower lysotracker fluorescence in cells treated with C1P. SiRNA mediated knock down of cPLA2 or pretreatment of cells with cPLA2 inhibitor arachidonyl trifluoromethyl ketone (AACOCF3) substantially prevented autophagosome accumulation in C1P treated H4 cells and rat cortical neurons. *In vivo,* we observed significant decrease in accumulation of autophagosomal marker LC3-II and its substrate p62 in the injured mouse cortex when mice were treated before and after CCI with AACOCF3. Taken together these data indicate that cPLA2 activation leads to lysosomal damage causing autophagosome accumulation in the cortex and contributing to neuronal cell death after TBI. Thus we propose, that inhibiting cPLA2 mediated lysosomal damage early after TBI may restore autophagosome clearance and decrease neuronal cell loss.

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Poster

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Title: Diazepam inhibits post-traumatic neurogenesis and blocks aberrant neuronal maturation

Authors: *L. E. VILLASANA[1], A. PETERS[2], R. MCCALLUM[2], G. WESTBROOK[3], E. SCHNELL[1,4];
[1]Anesthesiol. and Perioperative Med., [2]Sch. of Med., [3]Vollum Inst., Oregon Hlth. and Sci. Univ., Portland, OR; [4]VA Portland Hlth. Care Systems, Portland, OR

Abstract: Traumatic brain injury (TBI) robustly increases the generation of adult-born neurons within the dentate gyrus of the hippocampus, a brain region critical for learning and memory. Under healthy conditions, constitutive neurogenesis is important for certain forms of learning and memory, although whether post-traumatic neurogenesis is beneficial or harmful to cognitive restoration has yet to be established. Benzodiazepines, which are frequently administered to severely head-injured patients during hospitalization, target receptors known to modulate constitutive neurogenesis. To investigate whether benzodiazepine administration after TBI affects post-traumatic neurogenesis, we administered diazepam continuously for one week via implanted osmotic pump immediately after a controlled cortical impact (CCI) injury was performed on 2-month old mice. Diazepam administration attenuated the TBI-induced increase in hippocampal neurogenesis without reducing neurogenesis below baseline levels, and did not affect neurogenesis in uninjured control mice. Cell proliferation three days after injury as assessed by bromodeoxyuridine incorporation into dividing cell nuclei was unaffected by diazepam, suggesting that the inhibition of post-traumatic neurogenesis by diazepam may be attributed to changes in survival or differentiation of cells generated after CCI. Neuronal activity within the hippocampus, however, was significantly decreased as assessed by cFos immunohistochemistry. Diazepam exposure also mitigated the dendritic abnormalities previously reported in TBI-induced adult-born neurons, but did not inhibit the enhanced outward migration of new neurons through the granule cell layer. As diazepam selectively attenuated the increase in neurogenesis without affecting baseline levels of neurogenesis, it may be a powerful tool to determine whether the increased generation of neurons born after injury is beneficial or harmful to cognitive recovery.

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Poster

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Topic: C.09. Brain Injury and Trauma

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VA Merit Review B6570R and B78071

Title: Combined intervention using acute intrathecal baclofen (ITB) and locomotor exercise provides significant therapeutic outcome in reducing TBI-induced spasticity without adversely affecting cognitive, balance, anxiety, and activity performance

Authors: *P. K. BOSE[1,2,3], J. HOU[2], R. NELSON[1], G. MUSTAFA[2], J. WATTS[1], J. JOSEPH[1], S. TSUDA[2], L. PAGE[5], F. J. THOMPSON[1,2,4];
[1]North Florida/South Georgia VAMC, Gainesville, FL; [2]Physiological Sci., [3]Neurol., [4]Neurosci., Univ. of Florida, Gainesville, FL; [5]Medtronic Neuromodulation,Targeted Drug Delivery R & D, Medtronic Inc, Minneapolis, MN

Abstract: Spasticity is a major health problem for patients with moderate to severe traumatic brain injury (TBI). Following TBI, the onset of spasticity and its secondary muscular and orthopedic sequellae is rapid, and can begin as early as one week following injury. These progressively developing motor disabilities often represent significant barriers for re-entry of TBI patients into the community. However, current federal guidelines preclude the use of ITB therapy for spasticity treatment during the first year following TBI due to insufficient data to determine potential risk associated with early therapies on cognitive function, balance, and motor recovery. Moreover, it is not clear, if acute ITB is safe or provides a therapeutic advantage when combined with locomotor therapy. Therefore, the objective of this preclinical study was to determine the safety and effectiveness of an experimental rehabilitation program that combined ITB and locomotor exercise. In these studies ITB (Lioresal® baclofen injection; 0.8µg/hr) and treadmill locomotor exercise (Tm) were initiated at one week after TBI (450g / 1.5 m), and then continued for 4 weeks. We recently reported that enduring spasticity, balance, anxiety and cognitive deficits were observed and quantitated in this TBI model (Bose et al., 2013). Spasticity, anxiety-like behavior, balance, cognitive, and home caged performances were measured using an elevated plus maze (EPM), rotorod, Morris water maze (MWM), and Noldus Phenotyper, respectively. One month of ITB and Tm combined treatment produced the most significant reduction in spasticity without detectable impact on cognitive, balance, anxiety or activity recovery. In fact, this combined treatment completely blocked early onset spasticity. More importantly, this significant therapeutic benefit persisted even after cessation of ITB therapy. Interestingly, the combined therapy group exhibited significantly reduced MWM latency at the

fourth day of testing, and significantly less anxiety-like behavior in the EPM. Moreover, 12-hour video-tracked activity monitoring data (6pm through 6am) did not show any adverse effects. In fact, ITB+Tm group exhibited home caged behavioral patterns similar to normal animals. These observations indicated that initiating ITB at one week post-TBI was safe, feasible, and effective; and in combination with a Tm therapeutic exercise protocol provided the most effective rehabilitation. These studies also indicate that progressively, a broad spectrum of comprehensive data will reinforce confidence in the safety, feasibility, and efficacy of early intervention treatments with locomotor therapy for TBI-spasticity.

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Poster

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UTHSC Department of Anatomy

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Title: Mild traumatic brain injury in mice causes region specific deficits in oscillatory neuronal activity and functional connectivity that are rescued by the novel cannabinoid type-2 receptor inverse agonist SMM-189

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Abstract: Mild traumatic brain injury (mTBI) typically results from a closed-head insult after a primary blast shock wave, blow to the head, or head acceleration - deceleration during a collision. It is a frequent occurrence during military combat, sports, recreational activities, and vehicular accidents. MTBI can cause long lasting cognitive deficits. Depression and persevering fear are among the most commonly observed deficits in mTBI patients, and they have been reproduced in a focal cranial air-blast model of mTBI in mice (*1, 2*). Although these deficits strongly implicate neuropathology of the medial prefrontal cortex (mPFC) and the hippocampus, abnormalities in these brain regions after mTBI have not been confirmed. We used multi-electrode, multi-site recording techniques in the focal cranial air-blast mouse model of mTBI to investigate the effect of mTBI on oscillatory neuronal activity, coherence and phase-amplitude coupling (PAC) in medial prefrontal cortex (mPFC), the primary somatosensory and primary visual cortex (S1/V1), and the hippocampal CA1 area. We also investigated the effect of mTBI on CA1 sharp wave ripples (SWR) and on SWR evoked responses in the mPFC. Coherence and PAC of neuronal oscillations require precise temporal coordination of neuronal activity within and/or between regions, and we hypothesized that they would be sensitive indicators of cortical and hippocampal connectivity disruption following mTBI (*2, 3*). MTBI caused increased coherence of oscillatory activity at theta to beta (4-30Hz) frequencies within the mPFC and S1/V1, enhanced theta-gamma PAC within the mPFC, caused a downshift of SWR frequency in the CA1 and reduced SWR-evoked LFP activity in the mPFC. Systemic treatment of mTBI mice with the cannabinoid type-2 (CB2) receptor inverse agonist SMM-189 for two weeks after blast rescued most deficits. In the brain, CB2 receptors are selectively expressed on microglia, and SMM-189 acts to bias microglia from the pro-inflammatory M1 state to the pro-healing M2 state. Our findings link mTBI to specific deficits in oscillatory network activity and functional connectivity within mPFC, S1/V1 and hippocampus, and suggest targeting of neuroinflammatory processes as a promising route for the development of treatments for mTBI.
1. Guley, N.G., Rogers, J., Del Mar, N., Deng, Y., Islam, R.M., D'Surney, L., Ferrell, J., Deng, B., Hines-Beard, J., Bu, W., et al. (2016) J. Neurotrauma 33, 403-422.
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3. Reiner, A., Heldt, S.A., Presley, C.S., Guley, N.H., Elberger, A.J., Deng, Y., D'Surney, L., Rogers, J.T., Ferrell, J., Bu, W., et al. (2015). Int. J. Mol. Sci. 16, 758-787.

Disclosures: D.H. Heck: None. **S.S. McAfee:** None. **N.H. Guley:** None. **M.G. Honig:** None. **N. Del Mar:** None. **W. Bu:** None. **S.A. Heldt:** None. **B.M. Moore:** None. **A. Reiner:** None. **Y. Liu:** None.

Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 607.15/AA3

Topic: C.09. Brain Injury and Trauma

Support: Research Manitoba

Winnipeg Health Sciences Center

Title: Poly(ADP-Ribose) polymerase-1 causes mitochondrial respiratory dysfunction by regulating PGC-1α

Authors: ***P. LU**[1,2], A. KAMBOJ[1], S. CHOWDHURY[3], P. FERNYHOUGH[3,2], C. M. ANDERSON[1,2];
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Abstract: Excitotoxic oxidative stress and DNA damage causes excessive activation of the nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1). This leads to mitochondrial dysfunction and neuron death, but the mechanisms of PARP-1-induced mitochondrial damage are poorly understood. Peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) is a master regulator of mitochondrial biogenesis and respiration. PGC-1α activity is influenced by sirtuins, which are nicotinamide adenine dinucleotide (NAD^+)-dependent class III histone deacetylases. Since PARP-1 consumes NAD^+, we hypothesized that PARP-1 leads to mitochondrial dysfunction in neurons by inhibiting sirtuin activity and preventing support of mitochondrial function by PGC-1α. Primary cortical neuron cultures were exposed to the DNA alkylating agent, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) to initiate pathological PARP-1 activation. A Seahorse Flux Analyzer was then used to assess several facets of mitochondrial function. Beginning 4 hr after MNNG exposure, maximal respiratory rate, respiratory control ratio, and spare respiratory capacity were significantly reduced in a manner partially restored by PARP-1 inhibition (PJ34, 10 μM), PARP-1 deletion (*parp-1*$^{-/-}$ mice) or exogenous replenishment of NAD^+. MNNG caused PARP-1-dependent depletion of NAD^+ levels and commensurate inhibition of sirtuin deacetylase activity. Increased PGC-1α acetylation was also observed, corresponding with reduced PGC-1α binding to nuclear respiratory factor-1 (NRF-1), NRF-1 association with mitochondrial transcription factor A (TFAM) promoter, and TFAM expression. In agreement, mitochondrial DNA copy number and total mass were significantly attenuated by MNNG by a mechanism sensitive to PJ34 or exogenous NAD^+ replacement. Taken together, these data demonstrate that PARP-1 activation causes NAD^+ depletion severe enough to inhibit PGC-1α-directed mitochondrial mass and respiratory capacity in neuron cultures.

Disclosures: **P. Lu:** None. **A. Kamboj:** None. **S. Chowdhury:** None. **P. Fernyhough:** None. **C.M. Anderson:** None.

Poster

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Program#/Poster#: 607.16/AA4

Topic: C.09. Brain Injury and Trauma

Support: MOST 104-2923-B-038-001-MY3, Taiwan

Title: Pomalidomide mitigates neuronal loss, neuroinflammation and behavioral impairments induced by traumatic brain injury in the rat

Authors: ***J. WANG**[1], J.-Y. WANG[1], L.-Y. YANG[1], D. TWEEDIE[2], N. H. GREIG[2]; [1]Grad. Inst. of Med. Sci. TMU, Taipei, Taiwan; [2]Drug Design & Develop. Section, Translational Gerontology Branch, Intramural Res. Program,, NIA, NIH, Baltimore, MD

Abstract: Traumatic brain injury (TBI) is a risk factor for neurodegenerative diseases and mortality worldwide. The therapeutic effects and time window of pomalidomide (Pom), an FDA approved immunomodulatory agent, were evaluated in a moderate to severe rat model of TBI induced by controlled cortical impact. Pom (0.5mg/kg, i.v.) or vehicle was administered at 5 or 7 hr after TBI. Neurobehaviors were evaluated using swing test, adhesive removal, modified neurological severity scores and beam walking. Tissue levels of mRNA and protein of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β) were measured by reverse transcriptase-qPCR and ELISA, respectively. Our results indicate that post-injury treatment with a single Pom dose within 5 hr significantly alleviated functional impairments caused by TBI. Pom reduced the contision volume, augmented neuronal survival and provided anti-inflammatory effects. In addition, Pom concentration-dependently ameliorated glutamate-mediated cytotoxic effects, H2O2-induced oxidative stress on cell viability and reduced microglial cell activation in vitro. Our data suggest that Pom has therapeutic effects to improve histological and functional outcomes after TBI and reduces neuroinflammatory responses. These findings strongly support the further evaluation and optimization of Pom for potential use clinically in TBI. (supported by MOST 104-2923-B-038-001-MY3, Taiwan).

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Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

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Program#/Poster#: 607.17/AA5

Topic: C.09. Brain Injury and Trauma

Support: NIH Grants R01NS060005, R01HD069620, HD069620-S1, R01NS084967

Title: The antipsychotic drug aripiprazole benefits functional outcome after experimental brain trauma and does not attenuate the benefits of environmental enrichment

Authors: H. L. RADABAUGH, S. BESAGAR, M. J. LAPORTE, P. B. DE LA TREMBLAYE, C. O. BONDI, *A. E. KLINE;
Phys Med. & Rehab, Psych, Safar Ctr. Resuscitation Res., Univ. Pittsburgh, Pittsburgh, PA

Abstract: Introduction: The typical antipsychotic drug (APD) haloperidol (HAL), a D_2 receptor antagonist, has been shown to impede functional outcome after experimental traumatic brain injury (TBI). Furthermore, the deleterious effects persist for up to 3 months after drug withdrawal. Moreover, a recent study showed that HAL reduced the effectiveness of environmental enrichment (EE), a preclinical model of neurorehabilitation. Because agitation is common after TBI, patients are provided APDs so that they can be safely managed. However, many patients in rehabilitation will only experience agitation occasionally and thus will receive APDs intermittently. **Hypotheses**: Aripiprazole (ARIP), a partial D_2 and 5-HT_{1A} receptor agonist, will not impair recovery or reduce the effectiveness of EE regardless of whether administered once every day (i.e., chronic agitation) or once every other day (occasional agitation). **Methods**: Anesthetized adult male rats received a cortical impact of moderate severity or sham injury and were then randomly assigned to EE or standard (STD) housing. Treatments with ARIP (0.1 mg/kg; i.p.) or vehicle (VEH; 1.0 mL/kg; i.p.) began 24 hr after injury and continued once daily for 19 days, or once every other day for the same period. Motor (beam-walk) and cognitive (spatial learning) outcome were assessed on post-operative days 1-5 and 14-19, respectively. **Results**: Motor and cognitive function was significantly improved in the TBI+EE+VEH vs. TBI+STD+VEH group ($p<0.05$). Moreover, the TBI+EE+ARIP groups, regardless of dosing regimen, performed significantly better on all endpoints relative to the TBI+STD+VEH controls ($p<0.05$), but did not differ from one another or from TBI+EE+VEH ($p>0.05$). **Conclusions**: The data replicate previous work from our laboratory showing the EE improves functional outcome after TBI. Furthermore, ARIP, unlike HAL, did not impair recovery or reduce the efficacy of EE, which supports the hypothesis. **Significance**: ARIP is beneficial on its own and does not negate the benefits of rehabilitation (i.e., EE) and thus may be used to control TBI-induced agitation and aggression without compromising recovery.

Disclosures: **H.L. Radabaugh:** None. **S. Besagar:** None. **M.J. LaPorte:** None. **P.B. de la Tremblaye:** None. **C.O. Bondi:** None. **A.E. Kline:** None.

Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

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Topic: C.09. Brain Injury and Trauma

Support: NIH Grants

R01NS060005

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HD069620-S1

R01NS084967

Title: Chronic vs intermittent administration of the antipsychotic drugs haloperidol or risperidone after experimental traumatic brain injury: Insights into motor and cognitive recovery

Authors: *P. BARRA DE LA TREMBLAYE[1], L. J. CARLSON[1], M. J. LAPORTE[2], C. O. BONDI[1], A. E. KLINE[1];
[1]Physical Med. & Rehabil., [2]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Preclinical studies have shown that chronic administration of the antipsychotic drugs (APDs), haloperidol (HAL) or risperidone (RISP), impairs neurobehavioral recovery after TBI. However, APDs are often administered intermittently rather than continuously to TBI patients as restlessness and agitation occur irregularly during the recovery phase. The goal of this study was to test the hypothesis that intermittent (1 or 3 times per week) administration of HAL and RISP would produce less deleterious effects on motor and cognitive recovery compared to chronic (daily) administration. Anesthetized adult male rats received a controlled cortical impact (2.8 mm tissue deformation at 4 m/s) or sham injury and then were provided HAL (0.5 mg/kg; i.p.), RISP (0.45mg/kg; i.p.) or vehicle (VEH; 1 mL/kg; i.p.), with administration starting at 24 h after surgery and then either once daily (chronic), or 1 or 3 times weekly for 19 days. Beam-balance/walk and Morris water maze performance were assessed on post-injury days 1-5 and 14-19, respectively. The data revealed that the TBI groups administered HAL or RISP intermittently did not significantly differ from the VEH+TBI group in both motor and cognitive outcome, which is opposite to the chronic (once daily) treatment paradigm, which performed worse in all

behavioral measures compared to the VEH+TBI group ($p < 0.05$). These findings replicate previous reports that chronic administration of HAL or RISP negatively impact motor and cognitive recovery after cortical impact. Additionally, the data expands the research by demonstrating that intermittent administration of HAL or RISP, regardless of whether once or three times a week, did not impair recovery as those groups did not differ from the TBI+VEH-treated controls. The potential clinical implications of these findings suggest that intermittent administration of the APDs HAL or RISP may be safely used to control agitation in TBI patients, without negatively impacting subsequent recovery.

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Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

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Topic: C.09. Brain Injury and Trauma

Support: UPP/UPMC Academic Foundation (COB)

UPMC Rehabilitation Institute Pilot Program (COB)

NIH Grant HD069620 (AEK)

NIH Grant NS084967 (AEK)

NIH Grant NS060005 (AEK)

Title: Effects of chronic unpredictable stress on cognitive and depressive-like behaviors following experimental brain trauma

Authors: L. KUTASH, E. MCPEAKE, E. COOLEY, R. ROHAC, I. MARSHALL, J. CHENG, A. E. KLINE, *C. O. BONDI;
Safar Ctr. for Resuscitation Res., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Traumatic brain injury (TBI) affects 2 million individuals in the United States each year, and many survivors endure long-lasting cognitive impairments associated with frontal lobe disturbances, while also being vulnerable to neuropsychiatric disorders. Clinical and preclinical research has highlighted the importance of chronic stress, particularly when presented in an unpredictable fashion, as a major risk factor for many psychopathological conditions. In the

current study, we are beginning to assess clinically-relevant cognitive-behavioral and anxiety-like dimensions sensitive to both TBI and chronic unpredictable stress (CUS). We hypothesized that moderate TBI produced by controlled cortical impact (CCI) injury, as well as CUS exposure will render cognitive impairments in male rats in an attentional set-shifting test (AST), reduced sucrose preference and open field exploration, as well as blunted weight gain. Isoflurane-anesthetized adult male rats were subjected to a CCI (2.8 mm cortical tissue deformation at 4 m/sec) or sham injury over the right parietal cortex. Following surgery, rats were randomly assigned to receive CUS (21 days starting 5 days post-surgery) or 30 sec of handling (CTRL). Upon cessation of stress, rats were tested for perceived state of anxiety (open field test) and anhedonia (reduced preference of 1% sucrose-water versus regular water overnight). At 4 weeks post-surgery, rats were then tested on the AST, which involves a series of increasingly difficult discriminative tasks to obtain food reward. Preliminary results demonstrate that CUS exposure leads to a 5-10% weight gain reduction compared to CTRL group, yet the combination of TBI and CUS does not seem to negatively impact center exploration in the open field, or sucrose preference (n=8-12/group). We anticipate that TBI will lead to cognitive deficits, as previously reported (Bondi et al., 2014, *J Neurotrauma*), which will be further augmented by CUS exposure. This ongoing project will provide novel outcomes pertaining to cognitive capability, as well as anxiety- and depressive-like symptoms following overlapping chronic stress exposure and the recovery phase of TBI.

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Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

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Topic: C.08.Stroke

Support: Takeda Pharmaceutical Company

Title: Phosphodiesterase 10A inhibition promotes motor recovery in ischemic striatal stroke

Authors: S. Z. BIRJANDI[1], S. NAIR[2], K. SUZUKU[3], H. KIMURA[3], *S. CARMICHAEL[4]; [1]Neurol., David Geffen Sch. of Med. at UCLA, Los Angeles, CA; [2]Neurol., David Geffen Sch. of Med. at UCLAa, Los Angeles,, CA; [3]Takeda Pharmaceut. Co., Osaka, Japan; [4]UCLA Sch. Med., Los Angeles, CA

Abstract: Stroke is one of the leading causes of adult disability in the United States. Physical therapy leads to modest improvements in motor recovery, but there is currently no drug regimen available to enhance recovery after stroke. Phosphodiesterases (PDEs) are the enzymes that degrade the intracellular second messengers, cAMP and/or cGMP. Accumulating preclinical evidence suggests that PDE inhibitors promote learning and memory and attenuate disease progression in several neurologic illnesses. Among the PDE families, PDE10A is unique in its restriction to the basal ganglia/striatum, a common site of human stroke. The use of a PDE10A inhibitor has not been examined in recovery after stroke. Our goal was to test if a PDE10A inhibitor developed with demonstrated effect on brain function leads to improved motor recovery. We hypothesized that a PDE10A inhibitor would promote recovery in striatal but not cortical stroke. Using a murine model of striatal and cortical ischemic stroke, mice were given once-daily intraperitoneal injections of a novel PDE10A inhibitor TAK-063 for 9 weeks starting day 5 post stroke. Behavioral testing was evaluated during the course of drug treatment. After the 9-week drug regimen, the neuroanatomical tracer, biotinylated dextran amine, was microinjected into the motor cortex, and the motor cortical connections were mapped. Brains were also analyzed for relative degrees of gliosis and angiogenesis. In a parallel study, brains were harvested for measurement of brain-derived neurotrophic factor (BDNF), which is downstream of the cAMP signaling cascade. After treatment with 3 mg/kg of TAK-063, improvement in forelimb motor function and gait compared with stroke-only controls was exclusive to the striatal stroke model. No difference in behavior was observed in the cortical stroke model. Daily treatment with TAK-063 led to a significant increase in BDNF in the striatum when compared with stroke-only mice, normalizing BDNF levels to that seen in non-stroke mice. Differences in motor system connections (axonal sprouting) and gliosis and angiogenesis were not observed in either stroke model with TAK-063 treatment. Our findings demonstrate that treatment with TAK-063 has a differential effect on stroke recovery depending on stroke location, and studies are needed to examine potential therapeutic effects for striatial stroke in humans.

Disclosures: **S.Z. Birjandi:** 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; This study was sponsored by Takeda Pharmaceutical Company Limited. **S. Nair:** 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; This study was sponsored by Takeda Pharmaceutical Company Limited. **K. Suzuku:** A. Employment/Salary (full or part-time): Employee of Takeda Pharmaceutical Company. **H. Kimura:** A. Employment/Salary (full or part-time): Employee of Takeda Pharmaceutical Company. **S. Carmichael:** A. Employment/Salary (full or part-time): This study was sponsored by Takeda Pharmaceutical Company Limited.. 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; This study was sponsored by Takeda Pharmaceutical Company Limited..

Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 607.21/AA9

Topic: C.09. Brain Injury and Trauma

Support: The Ari and Regine Aprijaskis Fund, at Tel-Aviv University.

Title: Twincretin mitigates cognitive impairments and neuronal degeneration induced by mild traumatic brain injury in mice.

Authors: *C. G. PICK[1], M. BADER[2], V. RUBOVITCH[3], D. TWEEDIE[4], I. A. TAMARGO[4], R. D. DIMARCHI[5], K. TALBOT[6], N. H. GREIG[4];
[2]Dept. of Anat., [3]Anat., [1]Tel Aviv Univ., Tel Aviv, Israel; [4]Drug Design & Develop. Section Translational Gerontology Br., Natl. Inst. on Aging, NIH, Baltimore, MD; [5]Dept. of Chem., Indiana Univ., Bloomington, IN; [6]Dept. of Neurosurg., Cedars-Sinai Med. Ctr., Los Angeles, CA

Abstract: Traumatic Brain Injury (TBI) is an increasingly common event affecting 1.7 million people annually in the USA alone. TBI is a significant cause of death and disability in the developed world, particularly among young adults. At present, no effective pharmaceutical therapies for TBI are available. Mild TBI (mTBI) is a common neurological event, which accounts for some 80% to 90% of TBI cases. Victims of mTBI frequently manifest cognitive, behavioral and emotional difficulties that can be long lasting. Previous experiments in our laboratory have shown that mTBI may lead to short and long-term cognitive and behavioral deficits as well as apoptotic processes in the brains of mice. The incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are gastrointestinal hormones that induce glucose-dependent insulin secretion, promote β-cell proliferation and enhance resistance to apoptosis. GLP-1 mimetics are on the market as treatments for type II diabetes and are well tolerated. Both GLP-1 and GIP mimetics have shown neuroprotective properties in animal models of Parkinson's and Alzheimer's disease. Twincretin is a unimolecular, dual action co-agonist with balanced activity at both the GLP-1 and GIP receptors with optimized pharmacokinetics. In this study, we exposed male ICR mice to mTBI using a weight drop concussive head trauma device and administered twincretin in a 7-day regimen of subcutaneous injections starting 30 minutes after the injury. Subsequently, we investigated the effects of twincretin on the cognitive impairments and neuronal degeneration following mTBI. Twincretin ameliorated mTBI-induced visual and spatial memory deficits as measured in the novel object recognition paradigm and the Y-maze paradigm, respectively. These effects were observed at both 7 and 30 days post-mTBI. Using the elevated plus maze we assessed the anxiety-like behavior and well-being of the mice. There were no differences between the groups at both 7 and 30 days following mTBI. These findings show that the results of the novel object recognition and

Y-maze paradigms are not confounded by different levels of anxiety among experimental groups. In addition, neuronal loss was quantified immunohistochemically at 72 hours post-injury by the use of fluoro-Jade B and NeuN within the dentate gyrus on both sides of the brain. Twincretin demonstrated significant mitigation of mTBI induced neuronal degeneration in the dentate gyrus. These findings may offer a new potential therapeutic agent to treat the deficits caused by mTBI. Further studies will continue to investigate the neuroprotective effects and mechanism of action of twincretin.

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Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

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Program#/Poster#: 607.22/AA10

Topic: C.08.Stroke

Support: Hamamatsu Pharma Research, Inc.

Title: Comparison of tissue plasminogen activator (tPA) alone and in combination with thrombin inhibitor argatroban in a nonhuman primate model of thromboembolic stroke

Authors: J. SUZUKI[1], Y. ITANI[1], K. YAMANAKA[1], *A. HAMA[2], A. MATSUDA[1], H. TAKAMATSU[1];
[1]Hamamatsu Pharma Research, Inc., Hamamatsu, Japan; [2]Hamamatsu Pharma Res. USA, Inc., San Diego, CA

Abstract: When given early, tissue plasminogen activator (tPA) increases functional recovery following acute stroke by clot lysis and vascular recanalization. However, to achieve efficacy, there is a narrow therapeutic time window in which tPA must be administered. In addition, not all patients respond to tPA, which suggests the need for antithrombotics for the treatment of acute stroke with mechanisms other than that of tPA. Numerous promising treatments that showed significant efficacy in preclinical animal models of acute ischemic stroke have failed clinical trials. A potential limitation of current preclinical studies is the utilization of rodents as the model species. Nonhuman primates are phylogenetically closer to humans than rodents, which could narrow the current translational shortfall between preclinical and clinical findings. Thus, the goal of the current study was to uncover a possible beneficial effect of a combination of argatroban, a thrombin inhibitor, with tPA in a nonhuman primate model of thromboembolic ischemia. A thromboembolic stroke was induced in cynomolgus macaques by injection of blood

clots into the internal carotid artery. One hour following the induction of ischemia, either tPA (0.9 mg/kg) or vehicle was intravenously infused for 1 hour. After completion of tPA infusion, either argatroban (0.6 mg/kg) or vehicle was intravenously infused for 22 hours. Middle cerebral artery blood flow was monitored for 6 hours after ischemia. In addition, brain infarct volume (TTC staining) and functional assessment (Neurological Deficit Score; NDS) were determined 24 hrs after ischemia. Tissue plasminogen activator significantly decreased occlusion time, brain infarct volume and NDS compared to vehicle treatment. Preliminary findings suggest that macaques treated with the combination of argatroban and tPA showed decreased occlusion time, similar brain infarct volume and similar NDS compared to tPA alone-treated macaques. The current study in a nonhuman primate model suggests that the addition of argatroban could enhance recanalization rates, but does not further enhance tissue or functional recovery from a thromboembolic stroke compared to tPA treatment alone. Additional macaques will be tested to confirm the current findings.

Disclosures: **J. Suzuki:** A. Employment/Salary (full or part-time): Hamamatsu Pharma Research, Inc,. **Y. Itani:** A. Employment/Salary (full or part-time): Hamamatsu Pharma Research, Inc. **K. Yamanaka:** A. Employment/Salary (full or part-time): Hamamatsu Pharma Research, Inc. **A. Hama:** A. Employment/Salary (full or part-time): Hamamatsu Pharma Research, Inc. **A. Matsuda:** A. Employment/Salary (full or part-time): Hamamatsu Pharma Research, Inc. **H. Takamatsu:** A. Employment/Salary (full or part-time): Hamamatsu Pharma Research, Inc..

Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

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Program#/Poster#: 607.23/AA11

Topic: C.09. Brain Injury and Trauma

Support: NINDS P01NS045260-01

NINDS R21NS090397

Title: Calpain-1 and calpain-2 play opposite roles in traumatic brain injury (TBI) induced neurodegeneration

Authors: ***Y. WANG**, D. LOPEZ, J. TRAN, A. ALSTON, X. BI, M. BAUDRY;
Western Univ. of Hlth. Sci., Pomona, CA

Abstract: Traumatic Brain Injury (TBI) is a major cause of death and disability worldwide. The mechanism of injury varies and includes motor vehicle accidents, falls, sport injuries, and gunshot wounds, etc. Among the different types of TBI, penetrating traumatic brain injuries produce the worst outcomes and highest mortality rates. TBI induces neurodegeneration and axonal damage, and the calcium-dependent protease, calpain has been shown to be involved in these events. However, while calpain inhibitors have been tested in several animal models of TBI, there has not been any clinical trial testing the efficacy of calpain inhibitors in human TBI. One important reason for this, could be the lack of knowledge regarding the differential functions of the two major calpain isoforms in the brain, calpain-1 and calpain-2 (aka μ- and m-calpain). In this study, we used the controlled cortical impact (CCI) model in mice to test the specific functions of calpain-1 and calpain-2 in TBI-induced neurodegeneration. Immunohistochemistry with calpain activity markers performed at different time points after CCI in wild-type and calpain-1 KO mice showed that calpain-1 and calpain-2 were sequentially activated around the impact site. While calpain-1 was activated within 0-8 h after CCI, calpain-2 activation was not significant until about 8 h after CCI, peaked at 24 h and was still present at 72 h after CCI. TUNEL and Fluoro-Jade C staining showed enhanced neurodegeneration around impact site in calpain-1 KO mice after CCI, as compared to WT mice, suggesting that calpain-1 is neuroprotective. Systemic injection of a calpain-2 selective inhibitor, NA101, at 1 h or 4 h after CCI significantly reduced calpain-2 activity and neurodegeneration around the impact site, and reduced the contusion volume. Our data indicate that calpain-1 activity is neuroprotective while calpain-2 activity is neurodegenerative after TBI and that a selective calpain-2 inhibitor can reduce TBI-induced neurodegeneration.

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Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

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Topic: C.09. Brain Injury and Trauma

Support: NIH grant R01NS089901 (DZL)

NIH grant NS054652 (FRS)

Title: Combined inhibition of fyn and c-src reduces brain edema and improves cognitive function following traumatic brain injury in adult rats

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Abstract: Traumatic brain injury (TBI) is often accompanied by intracerebral and intraventricular hemorrhage. We have previously demonstrated that moderate TBI produced by lateral fluid percussion injury (LFPI) produces bleeding into the cerebrospinal fluid (CSF) in the ventricles, which activates Src family kinases (SFKs)[1]. In the current study we explored whether inhibition of SFKs could attenuate TBI-associated brain injury and behavioral deficits. Since several SFK subtypes including Fyn and Src have been reported in brain[2-4], we targeted these SFK subtypes using a newly developed *in vivo* nanoparticle-based siRNA transfection system to inhibit the specific SFK subtypes. One day after injections of nanoparticle-siRNAs (100μg, i.c.v.) for SFK family members Fyn and c-Src into the left lateral cerebral ventricle, Fyn and c-Src mRNAs decreased in rat hippocampus by 22% and 43%, respectively. As compared to vehicle treated TBI controls, the combined si-Fyn and si-c-Src decreased brain water content from 82.7% to 80.64% in ipsilateral cortex, and decreased sodium fluorescence concentration in brain homogenates from 2.4 to 1.5μg/ml ($p<0.05$) in the ipsilateral cortex at 24 hours after TBI. Additional studies are currently underway examining the effects of combined treatment with si-Fyn and si-c-Src on cognitive function assessed at 12 through 16 days after TBI using the Morris Water Maze, and on survival of hippocampal neurons ($NeuN^+$ cells) at 16 days after TBI. These data suggest that combined inhibition of Fyn and c-Src has the potential to improve outcomes following TBI. SFK inhibitors have been well tolerated in cancer patients in clinical trials and the FDA has approved the nanoparticle-based siRNA approach. We propose that combined inhibition of specific SFK subtypes Fyn and c-Src by nanoparticle-based siRNA might improve outcomes of patients with TBI. **Acknowledgements:** This study was supported by NIH grants R01NS089901 (DZL) and NS054652 (FRS). There were no conflicts of interest.

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Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

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Topic: C.08.Stroke

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NIH Grant R01NS046400 (SD)

Title: Hyperglycemia-mediated hematoma expansion during intracerebral hemorrhage is attenuated by PGD2 DP1 receptor antagonism

Authors: *A. S. AHMAD[1,2], M. MENDES[1,2], S. ALI[1,2], C. WASSERFALL[3,4], S. DORE[1,2,5]; [1]Dept. of Anesthesiol., [2]Ctr. for Translational Res. in Neurodegenerative Dis., [3]Dept. of Pathology, Immunol. and Lab. Med., [4]Diabetes Inst., [5]Departments of Neurology, Psychiatry, Pharmaceutics, Psychology, and Neurosci., Univ. of Florida, Gainesville, FL

Abstract: The respective importance of PGD2 and its receptor DP1 in the vasculature, the blood, and the brain warrants additional examination of their role in intracerebral hemorrhage (ICH) under hyperglycemic condition. In this study, we first tested the role of hyperglycemia on ICH, and, then, tested the therapeutic potential of DP1 receptor inhibition on functional and anatomical outcomes following ICH in hyperglycemic mice. Wildtype (WT) and DP1-/- C57BL/6 mice were given 2g/kg glucose and then blood glucose and C-peptide levels were monitored up to 3h after the injection. In the second cohort, WT and DP1-/- mice were subjected to hyperglycemia and ICH was induced at 1h after glucose injection by giving a single intrastriatal injection of collagenase to induce ICH. In the third cohort, WT mice were subjected to hyperglycemia and ICH, as described above, followed by treatment with saline or the DP1 receptor antagonist Laropiprant at 1h after ICH induction. Neurologic deficits, brain injury volume, and edema volume were determined at 72h. Furthermore, brain sections from these groups were subjected to Perls' staining and Iba1 and GFAP immunoreactivity to determine the effect of Laropiprant on the modulation of iron levels and gliosis. Acute injection of glucose led to a significant increase in the glucose level in WT and DP1-/- mice. DP1-/- mice exhibited faster clearance of glucose overload compared with the WT mice. Also, WT mice treated with Laropiprant also exhibited faster clearance of glucose. Similar patterns were observed in C-peptide levels of these groups. The injury volume in DP1-/- compared with WT was significantly lower (8.3±1.8 vs 19.3±5.6mm3; $p<0.01$). Moreover, WT mice treated with Laropiprant also exhibited significantly lower brain lesion volume (9.1±3.2 vs 19.3±5.6mm3; $p<0.01$). Interestingly, a significant decrease in Iba1 immunoreactivity and Perls'-labelled iron content was observed in hyperglycemic DP1-/- compared with the hyperglycemic WT mice. Together, the data suggest that blockade of the DP1 receptor improves glucose tolerance/clearance and ameliorate functional and anatomical outcomes following ICH in hyperglycemic condition. Ongoing studies are underway to further investigate optimal therapeutic conditions and mechanisms of action.

Disclosures: **A.S. Ahmad:** None. **M. Mendes:** None. **S. Ali:** None. **C. Wasserfall:** None. **S. Dore:** None.

Poster

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Topic: C.08.Stroke

Support: NIH Grant R01NS088084

Title: Manipulation of Ubiquilin-1 expression in the post-ischemic mouse brain to validate its role in neuroprotection following transient cerebral ischemic stroke

Authors: *Y. LIU[1], H. WANG[2];
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Abstract: Stroke is one of the most common causes of adult disability and death and is pathologically associated with oxidative stress, protein damage, and neuronal loss. We previously reported that overexpression of a ubiquitin-like protein, ubiquilin-1 (Ubqln), protects neurons against oxidative stress and ischemia-caused brain injury, while knockout of the gene exacerbates cerebral ischemia-caused neuronal damage and delays functional recovery. Although these observations indicate that Ubqln is a therapeutic target, transgenic manipulation-caused overexpression of Ubqln occurs before the event of ischemic stroke, and it remains unknown whether delayed Ubqln overexpression in post-ischemic brains within a clinically relevant time frame is still beneficial. To address this question, we generated lentiviruses either overexpressing mouse Ubqln or mouse Ubqln-shRNAs. Adult mice were subjected to middle cerebral artery occlusion (MCAO) for 90 minutes and then reperfused for 6 hours before intracerebral injection of the lentiviruses was performed. Mouse motor and sensory behaviors were evaluated at day 1, 3, 5 and 7 days following the ischemia/reperfusion. Our data indicate that post-ischemic overexpression of Ubqln significantly reduces neuronal injury and promotes functional recovery. In contrast, downregulation of Ubqln expression delays functional recovery and increases neuronal injury. To further understand the mechanisms underlying how Ubqln functions, we also isolated protein aggregates from the ischemia/reperfusion insulted mouse brains and found that Ubqln was present in the aggregates. These observations suggest that Ubqln may be a useful candidate for therapeutic intervention for stroke.

Disclosures: **Y. Liu:** None. **H. Wang:** None.

Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

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Program#/Poster#: 607.27/AA15

Topic: C.09. Brain Injury and Trauma

Support: NJCBIR CBIR11PJT012

Title: Oligodendrocyte response to mechanical injury: role of the Erk1/2 pathway

Authors: *J. KIM[1], P. GOKINA[1], A. ADAMS[1], B. J. PFISTER[2], H. A. KIM[1];
[1]Biol. Sci., Rutgers Univ., Newark, NJ; [2]Biomed. Engin., New Jersey Inst. of Technol., Newark, NJ

Abstract: Impact-accelerated forces to the head causes rapid stretching of the axons through out the brain termed traumatic axonal injury (TAI). Long myelinated axons within white matter tracts are especially vulnerable to the injury and accordingly, chronic myelin loss is a common occurrence after such traumatic brain injury (TBI). While axon pathology associated with TBI has been well elucidated, the molecular efficacy of mechanical force on myelin and oligodendrocyte is not fully understood. Using an in vitro TAI model, we investigated the effects of stretch injury on differentiated oligodendrocytes. Differentiated oligodendrocyte cultures were established on deformable silicone membrane, which was then stretched using computer-controlled air pressure. Within 24 hours after injury, oligodendrocytes at the site of injury lost membrane arborization and MBP expression or exhibited aberrant MBP accumulation. Cell death was not observed indicating that the injury response was induced in viable oligodendrocytes. Stretch injury also induced calcium-dependent Erk1/2 activation in differentiated oligodendrocytes. In vivo, experimental TBI on rat induced Erk1/2 activation in white matter oligodendrocytes within 4 hours after injury, suggesting that Erk1/2 may play a role in mediating the oligodendrocyte response to TBI. To directly assess the impact of Erk1/2 activation on myelin, we generated oligodendrocytes harboring a constitutive active mutant of MKK1 (CA-MKK1), which expression is induced by doxycycline. The CA-MKK1 oligodendrocytes were co-cultured with neurons and allowed to generate myelin, after which Erk1/2 was activated in oligodendrocytes with myelin by doxycycline. Erk1/2 activation resulted in myelin degeneration in viable oligodendrocytes and down regulation of myelin protein expression. In differentiated oligodendrocyte cultures, Erk1/2 activation induced loss of the differentiated phenotype, which was followed by the oligodendrocyte re-entry into the cell cycle. Altogether, our results suggest a role of Erk1/2 in mediating oligodendrocyte response and myelin loss following mechanical injury.

Disclosures: J. Kim: None. **P. Gokina:** None. **A. Adams:** None. **B.J. Pfister:** None. **H.A. Kim:** None.

Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

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Topic: C.08.Stroke

Support: MOHW grant/HI15C2796

NRF grant/2015R1A2A2A01004202

Title: Preclinical and clinical toxicity/safety profiles of sp-8203, a novel neuroprotectant for the treatment of cerebral ischemic stroke

Authors: *H. SONG[1], W.-K. KIM[1], J.-M. RYU[2], B.-S. KIM[2], G.-S. CHO[2], K.-S. BAE[3]; [1]Korea Univ. Med. Col., Seoul, Korea, Republic of; [2]Central Res. institute, Shin Poong Pharm. Co. Ltd, Ansan, Korea, Republic of; [3]Asan Med. Center, Univ. of Ulsan, Seoul, Korea, Republic of

Abstract: The novel neuroprotectant SP-8203 (Shin PoongPharm. Co. Ltd, South Korea) extends the therapeutic time window of tPA in embolic stroke rats by reducing the adverse effects caused by delayed tPA treatment. SP-8203 has successfully completed a Phase 1 clinical trial and is undergoing a Phase 2a clinical trial in ischemic stroke patients. The preclinical and clinical toxicity/safety profiles of SP-8203 are reported here. General toxicity tests in Sprague Dawley rats and Cynomolgus monkeys were performed by MPI Research (USA), Toxikon (USA) and Phase 1 clinical trial by Asan Medical center (Korea). The NOAEL of 4 week repeated dose of SP-8203 was 40 mg/kg/day in rats and 12 mg/kg/day in monkeys. There was no specific safety concerns noted in the preclinical program. A Phase I, single-center, randomized, double-blind, placebo-controlled study to assess the safety, tolerability, and pharmacokinetic of single ascending doses (SAD) and multiple ascending doses (MAD) of SP-8203 was conducted in healthy subjects. For the SAD assessment (n=48), cohorts of 8 subjects were randomly assigned to receive a single dose of either SP-8203 (n=6) or placebo (n=2). The doses were from 10 mg with incremental escalation up to 240 mg. For the MAD assessment (n=24), cohorts of 8 subjects were randomly assigned to receive one dose of either SP-8203 (n=6) or placebo (n=2) daily for 7 consecutive days, and the doses of SP-8203 were 30 mg, 60 mg, and 120 mg. Mild adverse events (AEs) occurred in 4 out of 48 subjects (8.3%) and no serious adverse events (SAEs) occurred, in the SAD assessment. Additionally, AEs were observed in 7 (29.1%) out of 24 subjects in the MAD again with no SAE, demonstrating the safety and tolerability of SP-8203.The pharmacokinetics of SP-8203 has been described in healthy subjects where no accumulation of SP-8203 was demonstrated with repeated dosing. SP-8203 was metabolically most stable in human. Taken together, the series of toxicity/safety assessments showed that SP-8203 is safe and well tolerated at the doses which will be used in the study of SP-8203 treatment

for the cerebral ischemic stroke in man. A Phase 2a proof-of-concept study is underway to evaluate the safety and exploratory efficacy of SP-8203 in combination with tPA in patients with acute ischemic stroke. (HYS and W-KK equally contributed)

Disclosures: **H. Song:** None. **W. Kim:** None. **J. Ryu:** None. **B. Kim:** None. **G. Cho:** None. **K. Bae:** None.

Poster

607. Pharmacological Strategies to Prevent Injury from Trauma and Stroke

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Program#/Poster#: 607.29/AA17

Topic: C.08.Stroke

Support: Department of Science and Technology

Title: Estrogen reduces stroke induced brain damage by differentially altering ASIC1a and 2a expression

Authors: ***M. MUKHOPADHYAY**[1], A. WAHUL[2], P. JHELUM[2], S. CHAKRAVARTY[2], A. BERA[1];
[1]Indian Inst. of Technol., Chennai, India; [2]CSIR-Indian Inst. of Chem. Technol., Hyderabad, India

Abstract: Activation of acid sensing ion channels (ASICs) during cerebral ischemia has been implicated in stroke induced brain damage. Estrogen is known to be neuroprotective against many neurological disorders and stroke. We hypothesise the involvement of ASICs in the estrogen mediated neuroprotection against stroke. We found that while ASIC1a exacerbates ischemic cell damage, ASIC2a subtype is neuroprotective. Increase of ASIC2a expression possibly increases the population of ASIC1a and 2a containing heteromeric channels which have much lower activation pH than homomeric ASIC1a. Thus, shifting of pH_{50} to lower values renders the channel ineffective at the moderate drop of extracellular pH during ischemia. We detected an up-regulation of ASIC2a levels in female mice with intact ovaries following experimental stroke, whereas the levels of ASIC1a went down. This pattern however was not seen in male and ovariectomised mice. Estrogen supplementation in ovariectomised mice reinstated the ASIC2a levels and decreased ASIC1a. This substantiates our hypothesis that estrogen exhibits neuroprotective properties by regulating ASICs.

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Poster

608. Brain Stimulation and Recovery After Stroke

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Program#/Poster#: 608.01/AA18

Topic: C.09. Brain Injury and Trauma

Support: NINDS 5R01NS086422

DARPA SUBNETS

Title: Enhanced synaptic plasticity in a brain injury model treated with phasic striatal deep brain stimulation

Authors: *H. KATNANI[1], J. ARONSON[2], E. ESKANDAR[2];
[1]Dept. of Neurosurg., [2]Massachusetts Gen. Hosp., Boston, MA

Abstract: We have previously demonstrated that striatal deep brain stimulation (DBS) can enhance functional recovery of mice in a sub-acute phase of traumatic brain injury. We found that brain injured mice, receiving precisely timed stimulation at the reinforcement period of the task, performed significantly better then sham mice on a spatial memory task after 2 days and also reached the same performance level of uninjured mice after 6 days. With the observation of changes in performance occurring on a relatively fast time-scale, we evaluated dynamic neural mechanisms with a short time-constant. Converging evidence suggests that spatial memorization during the reinforcement period relies on early activation of synaptic receptors, which induces structural modifications in pre- and post-synaptic neurons that alters synaptic function. Accordingly, immunohistochemical analysis from our study revealed that stimulated mice had upregulated levels of synapsin-1 and GAP43. The promotion of these proteins with striatal stimulation could therefore be enhancing signal propagation through the striatum and hippocampus, leading to enhanced spatial memory function. In line with this, we utilized molecular biology techniques across multiple control groups to further assess modification of synaptic proteins from punch outs of the striatum, prefrontal cortex, and hippocampus, elucidating if DBS in the striatum is important to altering synaptic plasticity across multiple loci.

Disclosures: **H. Katnani:** None. **J. Aronson:** None. **E. Eskandar:** None.

Poster

608. Brain Stimulation and Recovery After Stroke

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Topic: C.08.Stroke

Support: The Stroke Association project grant TSA 2013-09

King's College London doctoral scholarship

Title: Improvement in upper limb function after unilateral, but not bihemispheric, transcranial direct current stimulation.

Authors: *M. K. FLEMING[1], J. C. ROTHWELL[2], L. SZTRIHA[3], J. T. TEO[3,2], D. J. NEWHAM[1];
[1]Ctr. of Human and Aerospace Physiological Sci., King's Col. London, London, United Kingdom; [2]Inst. of Neurol., Univ. Col. London, London, United Kingdom; [3]Dept. of Stroke & Neurol., King's Col. Hosp. NHS Fndn. Trust, London, United Kingdom

Abstract: Transcranial direct current stimulation (tDCS) is a safe and non-invasive brain stimulation technique with the potential to improve upper limb function after stroke. Based on the interhemispheric competition model, ipsilesional primary motor cortex (M1) excitability can be increased with anodal tDCS, contralesional M1 excitability can be decreased with cathodal tDCS or both can be used simultaneously (bihemispheric). The impact of these different electrode arrangements on the efficacy of tDCS, and whether changes are due to callosal connections between cortices, is unclear. This study therefore aimed to investigate the effect of tDCS electrode arrangement on within-session change in upper limb function in chronic stroke survivors and change in transcallosal inhibition (TCI). 24 stroke survivors (range 3 - 124 months post-stroke, 34 - 81 years of age) with persistent upper limb impairment received 20 minutes of 1 mA tDCS while performing a motor sequence learning task. This involved reaching movements with the paretic arm to targets on a monitor in a repeated order. Four tDCS conditions were studied in a crossover design; i) anodal to the ipsilesional M1, ii) cathodal to the contralesional M1, iii) bihemispheric and iv) sham. Upper limb function was assessed before and after tDCS, using the Jebsen-Taylor hand function test (JTT). Changes in TCI were assessed as the duration of the ipsilateral silent period, using transcranial magnetic stimulation. Performance of the motor sequence learning task was unaffected by tDCS. There was a significant effect of tDCS condition on upper limb function as unilateral tDCS (anodal or cathodal) improved JTT time compared to sham ($p < 0.05$), but bihemispheric did not (Fig. 1). There was no effect on TCI ($p > 0.5$), and no relationship between changes in TCI and upper limb function. This indicates that unilateral, but not bihemispheric, tDCS improves upper limb function but that the response to tDCS does not

appear to be driven by changes in TCI. These results have implications for the use of tDCS during upper limb rehabilitation after stroke.

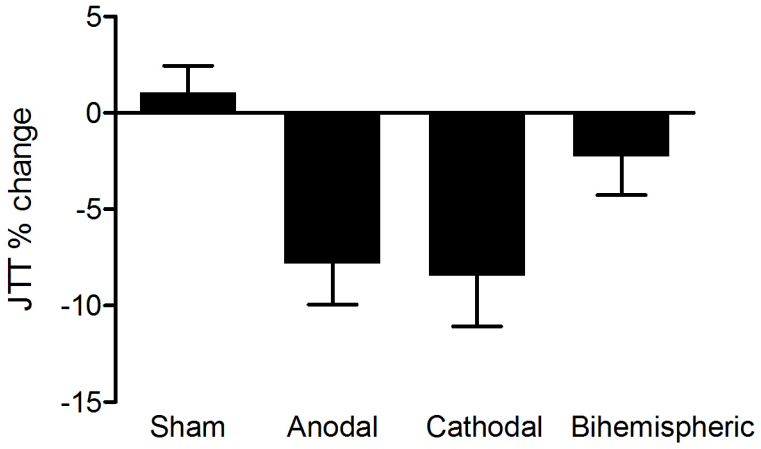

Fig. 1. Percentage change in JTT time for each tDCS condition.

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Poster

608. Brain Stimulation and Recovery After Stroke

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 608.03/BB2

Topic: C.08.Stroke

Support: University of Minnesota Program in Physical Therapy Departmental Funds

Title: Contralesional rtms and motor training in a single subject with brainstem stroke

Authors: *K. FROST, T. BROBACK, N. CARLSON, C. DAGGETT, M. DALBEC, J. R. CAREY;
Univ. of Minnesota Syst., Minneapolis, MN

Abstract: Background: Repetitive transcranial magnetic stimulation (rTMS) coupled with motor training may improve post-stroke motor recovery. The influence of rTMS on ipsilesional corticospinal excitability is usually assessed using ipsilesional motor evoked potentials

(IpMEPs). However, nearly 40% of individuals with stroke do not have an elicitable IpMEP and are excluded from study samples. Thus, the efficacy of rTMS in this population is unknown. This study explores the influence of primed rTMS in a single subject with no baseline IpMEP amplitude ≥50 μV through the use of functional magnetic resonance imaging, transcranial magnetic stimulation and motor function tests. Methods: The human subject (45 year old male, six years post left pontine stroke) received two randomly applied interventions in a cross-over design with a one-month washout. *Active rTMS+training* included active 6-Hz priming of active 1-Hz principle rTMS followed by one hour of paretic finger tracking training. S*ham rTMS+training* included sham 6-Hz priming of sham 1-Hz principle rTMS to the contralesional primary motor area (M1) followed by one hour of paretic finger tracking training. Outcome measures were M1 and primary sensory area (S1) laterality indices, the probability of eliciting an IpMEP following single pulse and interhemispheric inhibition protocols, box-and-block score and finger tracking score. Due to the single-subject nature of this study, visual analyses were used for all outcomes. Results: *Following active rTMS+training,* the M1 laterality index moved closer to zero, reflecting a trend toward decreasing activation of contralesional M1. The S1 laterality index shifted from positive to negative during non-paretic finger tracking. Box-and-block and finger tracking scores did not change. *Following sham rTMS+training*, the M1 laterality index shifted from negative to positive, reflecting greater cortical activation of ipsilesional M1. The S1 laterality index, box-and-block and finger tracking scores did not change. For both interventions, the probability of eliciting an IpMEP decreased. Conclusions: Neural activation changed its laterization from contralesional to ipsilesional M1 following five days of paretic finger tracking without adjunctive rTMS (i.e. with sham rTMS). However, there was no accompanying functional improvement, possibly because of the short training time. Including active rTMS as an adjunct to motor training did not improve motor function or likelihood of eliciting an IpMEP. We conclude that there was no benefit of coupling contralesional rTMS with motor training in this single subject with no elicitable IpMEP ≥50 μV.

Disclosures: K. Frost: None. **T. Broback:** None. **N. Carlson:** None. **C. Daggett:** None. **M. Dalbec:** None. **J.R. Carey:** None.

Poster

608. Brain Stimulation and Recovery After Stroke

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Program#/Poster#: 608.04/BB3

Topic: C.08.Stroke

Support: ERA-NET NEURON

Title: Deep brain stimulation of the entorhinal cortex rescues memory in a rat model of global ischemia

Authors: *E. GONDARD[1], L. TEVES[1], C. HAMANI[1,2], S. K. KALIA[1], M. TYMIANSKI[1], A. M. LOZANO[1];
[1]Neurosurg., Toronto Western Hosp. - Univ. of Toronto, Toronto, ON, Canada; [2]Res. Imaging Ctr., Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada

Abstract: Deep brain stimulation (DBS) is a well-established therapeutic modality for the treatment of movement disorders. Stroke is among the most frequent cause of disability in adults worldwide and often results in long-term neurological deficits in various functions including memory. Recently, the potential beneficial effects of DBS have been reported for memory enhancement in rodents and humans. Despite these promising results, DBS has not yet been tested for its ability to improve memory impairments following global ischemia. Using stimulation parameters analogous to clinical high-frequency DBS, we hypothesized that stimulating the neural circuits that underlie learning and memory might improve memory impairments observed in a rat model of global ischemia (GI). Two weeks after the GI induction, rats were bilaterally implanted with electrodes in the entorhinal cortex (EC) and underwent high frequency stimulation for 1 hour. Six weeks after DBS treatment, rats were assessed for changes in locomotor activity in the Open Field and learning and memory using the Morris water maze (MWM). Four different groups were studied: sham-stroke animals with DBS off (CTL-CTL), sham-stroke animals that received DBS (CTL-DBS), GI rats with DBS off (GI-CTL) and GI rats that received DBS treatment (GI-DBS). No differences were observed in the Open Field, suggesting that GI and DBS did not affect locomotion. GI led to a dramatic loss of neurons in the CA1 subregion of the hippocampus, which are known to be essential for spatial learning and memory. GI-CTL rats exhibited memory impairments when tested in the Morris Water Maze. However, when treated with DBS, GI rats crossed the platform area significantly more and spent more time in the quadrant area than GI-CTL rats, suggesting that the memory impairment caused by GI was improved with DBS treatment. Moreover, there was no difference between GI-DBS and CTL-CTL or CTL-DBS groups during the probe test, indicating that DBS of the EC can rescue some memory deficits induced by global ischemia. These results indicate that DBS may be a potential new approach to treat patients suffering from stroke.

Disclosures: **E. Gondard:** None. **L. Teves:** None. **C. Hamani:** None. **S.K. Kalia:** None. **M. Tymianski:** None. **A.M. Lozano:** None.

Poster

608. Brain Stimulation and Recovery After Stroke

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Topic: C.08.Stroke

Support: HRC Project Grant 14/136

HRC PhD Scholarship (AM)

Title: Revisiting interhemispheric imbalance in chronic stroke: a tDCS study

Authors: *W. D. BYBLOW[1,2], A. B. MCCAMBRIDGE[3], J. W. STINEAR[2];
[2]Exercise Sci. and Ctr. for Brain Res., [1]Univ. of Auckland, Auckland, New Zealand; [3]Grad. Sch. of Hlth., Univ. of Technol. Sydney, Sydney, Australia

Abstract: At the chronic stage of stroke patients with moderate-severe upper limb (UL) impairment rely on descending commands from the contralesional motor cortex (M1) for controlling both ULs. Although this may seem maladaptive, recent studies have shown that down-regulation of contralesional M1 excitability produces neurophysiological or behavioral deficits. We examined whether anodal transcranial direct current stimulation (a-tDCS) would increase contralesional M1 excitability and improve paretic UL coordination in a circle drawing task. We also explored associations between behavioural change scores and potential biomarkers derived from clinical, neurophysiological and neuroimaging variables. Patients were clinically assessed on measures of UL impairment, function and spasticity, then received a-tDCS, cathodal (c-tDCS) or sham stimulation in randomised order across separate sessions. The target electrode was positioned over contralesional M1 (1 mA, 20 cm^2, 15 min). Contralateral and ipsilateral corticomotor pathway excitability was examined using transcranial magnetic stimulation and motor evoked potentials (MEP). Fractional anisotropy within the posterior limbs of the internal capsules, and basal gamma-amino butyric acid (GABA) concentration in each M1, were assessed with magnetic resonance imaging (30 diffusion directions, b=2000) and spectroscopy (MEGA-PRESS) respectively at 3T. For the group, chronicity was 113 (14-192) months, impairment on the UL Fugl-Meyer scale was 27 (9-58, max 66), Action Research Arm Test scores were 21 (0-57, max 57) and spasticity on the Modified Ashworth Scale ranged from 1-3. Both contralateral ($P = 0.017$) and ipsilateral ($P = 0.092$) MEPs were facilitated after a-tDCS, and accompanied by a trend toward improved circle drawing (aspect ratio, AR) with the paretic UL ($P = 0.085$). The effect of a-tDCS on AR was associated with chronicity ($R = 0.88$, $P = 0.002$) and spasticity ($R = 0.70$, $P = 0.036$) such that those with greater chronicity and spasticity achieved the most benefit from contralesional a-tDCS. The effect of c-tDCS on AR was associated with baseline motor performance ($R = 0.80$, $P = 0.01$) and GABA:Cr ratio within ipsilesional M1 ($R = 0.75$, $P < 0.013$). Contralesional c-tDCS did not suppress corticomotor excitability ($P > 0.24$) or influence

AR (all $P > 0.80$). These findings indicate that further increasing contralesional excitability may benefit some patients. This study identifies potential biomarkers that may be useful for identifying patients who are suitable for contralesional tDCS.

Disclosures: W.D. Byblow: None. **A.B. McCambridge:** None. **J.W. Stinear:** None.

Poster

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Topic: C.08.Stroke

Support: AHA 15PRE24920006

AHA 14GRNT20460001

NIH K01 HD060886

Great Lakes Scholarship Program

BGE Coca-Cola Scholarship Program

Title: The contribution of intact hemisphere dorsal premotor cortex to paretic arm motor performance after severe stroke

Authors: *R. HARRINGTON[1], E. CHAN[2], A. K. ROUNDS[3], C. J. WUTZKE[4], A. W. DROMERICK[5,6], P. E. TURKELTAUB[6,5], M. L. HARRIS-LOVE[3];
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Abstract: Stroke affects almost one million Americans every year and many are left with long-term effects. One such effect is chronic post-stroke arm impairment, which can drastically affect independence in activities of daily living and quality of life. Existing treatments are of limited efficacy, particularly for patients with relatively severe post-stroke arm impairment. We examined how transcranial magnetic stimulation (TMS) perturbation of several sites within the intact hemisphere [dorsal premotor cortex (PMd), primary motor cortex (M1), and dorsolateral prefrontal cortex (DLPFC)] during a reaching task affected reaching performance. Chronic stroke patients who had not recovered active wrist or finger extension in the affected arm, but

had partial shoulder/elbow movement, participated in the study (n=15). Patients were instructed to, upon seeing a visual 'Go' signal, reach forward as quickly as possible with the affected arm to contact a target placed at approximately waist height and oriented in the horizontal plane. TMS (double-pulse; ISI 25 ms; 120% of resting motor threshold for unaffected biceps) was delivered over M1, PMd, or DLPFC of the intact (i.e. ipsilateral) hemisphere during the reaction time period between the 'Go' signal and the onset of movement. Analysis of hand-path kinematics in trials with vs. without TMS perturbation revealed that perturbation of PMd resulted in a significantly greater reduction in speed of reaching (movement time) than occurred with perturbation of M1 or DLPFC ($p<0.05$). Analysis of hand path parameters showed that stimulation of PMd, but not M1, resulted in a longer overall trajectory of movement ($p<0.05$), a greater decrease in the smoothness of the movement ($p<0.05$), and a larger increase in endpoint error ($p=0.05$). Taken together, these results suggest that PMd of the intact hemisphere can contribute to the speed and trajectory of affected arm reaching movements in patients with severe arm impairment.

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Poster

608. Brain Stimulation and Recovery After Stroke

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Title: High-frequency rTMS improves functional recovery and promotes neurogenesis through BDNF signaling pathways in a rat model of ischemic stroke

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Abstract: Objective: This study was performed to investigate whether high-frequency rTMS can improve functional recovery or promote neurogenesis, and to examine the role of BDNF-TrkB pathways in high-frequency rTMS-induced effects in a rat model of middle cerebral artery occlusion (MCAO).
Methods: A total of 56 adult Wistar rats with MCAO operation were randomly divided into four groups: 20Hz group (n=16), iTBS group (n=16), control group (Sham stimulation group, n=16) and sham-operated group (n=8, filament was not inserted into the artery). The rats were sacrificed 7 or 14 days after evaluating the neurological function. In addition, neurogenesis around the peri-infarction region was examined with immunecytochemical stainings of several specific markers, including Ki67,Nestin,DCX,CD31 and NeuN. Some brain tissues were also used to detect protein levels of BDNF and TrKB with Western blots.
Results: The results showed that 20Hz rTMS and iTBS significantly improved neurological function ($p<0.05$) and reduced infarct volume ($p<0.05$). Moreover, both treatments promoted neurogenesis as evidenced by the increased Ki67/Nestin-positive and Ki67/DCX-positive cells in ischemic hemisphere. These beneficial effects were in conjunction with significant elevated BDNF and TrkB protein levels.
Conclusions: High-frequency rTMS improves functional recovery and promotes neurogenesis possibly through BDNF signaling pathways in ischemic rats.

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Poster

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Topic: C.08.Stroke

Support: NIH RO1HD061363

Title: Activity dependent stimulation of the lateral cerebellar nucleus to promote post-stroke motor improvement

Authors: *C. WATHEN[1], J. COOPERRIDER[2], H. CHAN[2], H. PARK[2], K. BAKER[2], J. GALE[2], A. MACHADO[2];
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Abstract: Deep brain stimulation (DBS) of the lateral cerebellar nucleus (LCN) has been shown to improve motor recovery after ischemic stroke in preclinical models when stimulation is delivered chronically, independent of motor behavior, during the animal's active period. We hypothesize that efficacy may be improved further by pairing stimulus delivery to specific epochs in relation to a rehabilitative motor task. The goal of the present study was to develop a task that would enable activity dependent stimulation in a rodent model. Long-Evans rats were trained on a modified version of the isometric pull task. Rats were trained to wait in front of a slot with the forepaw resting on a capacitive plate before reaching through the slot to grasp and pull a stationary handle. In the first stage of training, with the handle retracted fully out of reach, rats were trained to activate the capacitive plate for progressively longer periods of time prior to receiving a reward. Next, the handle was introduced inside the cage and the rats received a reward only after activating the capacitive plate for a sufficient period of time before interacting with the handle. In subsequent stages, the handle was retracted step-wise out of the cage to its final position and the force threshold required to receive a reward was adaptively increased. Once trained to a pre-specified criterion, rats underwent stroke induction via endothelin-1 injection in the primary motor cortex and electrode implantation in the contralesional LCN. Animals were assigned to receive no stimulation, continuous stimulation, or activity-dependent stimulation (initiated when the rat lifted its forepaw from the capacitive plate and terminated upon handle release). Assessments were performed over a period of four weeks. Sixteen rats were trained on the task. Training to criterion required 37.75±1.2 days (Mean ± SEM). The first stage of training on the capacitor lasted 21±2 days. After introducing the handle, the time required to reach criterion for stroke induction was 16.75±0.5 days. Four animals were excluded and did not undergo stroke induction; 2 for failure to meet criterion, and 2 for interacting with the capacitor with the nose, rather than the forepaw. Following a two week post-stroke recovery period, animals successfully resumed execution of the task. Rats were successfully trained on a multi-step behavioral task that enabled delivery of activity-dependent stimulation. Ongoing studies are aimed to determine if DBS applied only during a critical period of reaching behavior is capable of improving post-stroke motor impairment.

Disclosures: C. Wathen: None. **J. Cooperrider:** None. **H. Chan:** None. **H. Park:** None. **K. Baker:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IntElect Medical, ATI, Cardionomics. **J. Gale:** None. **A. Machado:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Enspire, Boston Scientific, ATI, Functional Neuromodulation, Cardionomics.

Poster

608. Brain Stimulation and Recovery After Stroke

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Topic: C.08.Stroke

Support: CNPq 200970/2015-1

NIH R01HD061363

Title: A method for the quantification and visualization of stroke volume lesion using MRI

Authors: *L. COVOLAN[1,2], C. WATHEN[2], J. COOPERRIDER[2], H. BATTAPADY[2], J. CHEN[2], C. ANDROJNA[2], A. MACHADO[2];
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Abstract: Determination of stroke volume is an important parameter to be taken into consideration while evaluating behavioral performance during ongoing experiments, such as those in rodent models. The methods we describe below would allow for the quantification and visualization of an ischemic lesion using only free, open source software. Ischemic stroke was induced in a Long-Evans rat by the injection of endothelin-1 in the vicinity of the middle cerebral artery. Several days after stroke induction the subject died, at which time the brain was extracted and preserved in 4% paraformaldehyde. Following preservation, the brain was imaged with the Bruker Biospec 70/20 7T MRI system using the mouse brain array coupled with the standard 86 mm coil (Bruker Corp). Axial, three-dimensional T2 Turbo RARE images were acquired using the following parameters: TE = 40 ms, TR = 1800 ms, SA=1; Image size = 150x150x150 and resolution = 0.173x0.173x0.173 mm. Following image acquisition, stroke volume was calculated using ImageJ (NIH). The image was first aligned within the standard viewing planes and resized. Next, the stroked hemisphere of the brain was reflected across the midsagittal plane. Using the image calculation tool, the two hemispheres were overlaid by subtracting the stroked hemisphere from the healthy hemisphere. Outside of the lesion, this resulted in a null image due to the subtraction of voxels of similar intensity. In the ischemic lesion, however, significant atrophy had taken place resulting in a low intensity signal which did not appreciably change the intensity of the corresponding healthy hemisphere following image subtraction. As a result, in the image resulting from the subtraction of the healthy and stroked hemispheres, the remaining signal corresponded to the stroke lesion volume. The resulting stroke volume image was then saved in the Analyze file type. Volview 3.4 (Kitware Inc, Clifton Park, NY) was then used to visualize the resulting stroke volume. The original T2 Turbo RARE image was first loaded. Next, using the “Merge Volumes” utility, the stroke volume was merged with the original image. False coloring of the stroke volume then allowed for visualization of the

stroke volume in both 2D and 3D. Further refinement of the stroke volume measurement is underway with the implementation of (a) slice-by-slice co-registration, (b) acquisition of higher resolution data sets (or images), and (3) evaluation of alternate MRI sequences (e.g. T1_FLASH_3D).

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Poster

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Topic: C.08.Stroke

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Title: Behavioral assessment using the pellet tray task in a rodent model of ischemia

Authors: *J. COOPERRIDER, C. WATHEN, A. LIU, H. CHAN, J. T. GALE, K. B. BAKER, A. G. MACHADO;
Cleveland Clin., Cleveland, OH

Abstract: The availability of sensitive and meaningful tools to measure functional recovery after neurological disease is critical to the success of preclinical neurorehabilitation models. The pasta matrix task is a commonly utilized task that our lab has used to measure the rehabilitative effect of deep brain stimulation of the lateral cerebellar nucleus after stroke. Here, we describe a complementary task to the pasta matrix that was designed to require a similar motor pattern, while presenting a greater challenge to the animal and compare it to the more established pasta matrix task. Rats were trained on both the pasta matrix task and a lab-built pellet tray task. Both tasks require the animal to reach through a narrow aperture to grasp and retrieve pasta or pellets with the paw contralateral to the presented food. Pasta is presented upright in a grid pattern and must be broken to be retrieved, while pellets must be grasped and retrieved without dropping. Individual performance in naïve animals was significantly correlated between the tasks. Animals then underwent a small, endothelin 1-induced cortical ischemic injury. Following stroke, rats showed a significantly greater deficit on the pellet tray task compared to the pasta matrix task across the 15 subsequent post-stroke assessment days, and performance on the two tasks was no longer correlated. The pellet tray task can be constructed from easily attainable and inexpensive components and provides a sensitive supplemental or alternative measure of post-injury motor deficit.

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Poster

608. Brain Stimulation and Recovery After Stroke

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Topic: C.08.Stroke

Title: Activation of ventral attention network by transcranial direct current stimulation for a patient with unilateral spatial neglect

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Abstract: Unilateral spatial neglect (USN), a common neurological syndrome following right hemispheric lesions, has become evident as a widespread attention network disorder. We previously revealed hyperactivity of the frontal cortex as a neural mechanism underlying the compensatory strategy during recovery process after USN. While an optimal level of compensatory strategy is necessary to avoid neglect behavior in daily life, excess attention bias toward neglected space may lead adverse effects, such as reduced tolerance and suppression of the bottom-up attention. The subject of this case study was a 42-year-old man who had suffered extensive infarction of the right hemisphere by the obstruction in internal carotid artery 10 months earlier. In spite of a severe neglect in the daily living, the patient gradually recognize his neglect behavior. At the participation of this study, he clearly had excess attention bias toward neglected space. In order to optimize an involvement of compensatory strategy, we here attempted to apply combined intervention of temporo-frontal transcranial direct current stimulation (tDCS) and visual stimuli on PC display. We hypothesized that neuromodularoty intervention of tDCS induce elicitation of ventral attention network, and might result a reduction of the excess frontal activity. Anodal and cathodal electrodes were placed at right temporal and

frontal cortex, and the stimulus intensity was set to 2mA. Based on the BAB design (two weeks of each phase), the B/A phase consists of visual stimuli real/sham tDCS for 20 minutes in a session. In order to evaluate an extent of gaze and it's cortical mechanisms, EEG was recorded when the patient performed eye pursuit-based choice reaction task. At the beginning of intervention, the patient clearly showed extensive leftward gaze shift which accompanied with the increased in theta-band power in the frontal cortex even at rest. Interestingly, degrees of leftward gaze shift was improved after neuromodulatory intervention, and these alteration accompanied the reduction of frontal theta-band power. The present results imply that task-dependent and tDCS-induced elicitation of the ventral attention network might activate bottom-up attention and then effectively work to reduce excess frontal activity.

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Poster

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Topic: C.08.Stroke

Title: Analysis of visual search pattern during free viewing of horizontally flipped image: Novel approach for the evaluation of visuospatial neglect

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Abstract: Eye tracking has been regarded as one of effective tool for identifying the behavioral aspect of unilateral spatial neglect (USN) which is a common neurological syndrome following right hemisphere lesion. In the present study, we attempted to develop an intuitive evaluation for USN behavior with the analysis of gaze distribution during free viewing of a pair of horizontally flipped images. 26 patients with right hemisphere damage participated in this study, and was divided into with and without neglect symptom (USN group: n=12, RHD group: n=14) based on the score of behavioral inattention test (BIT). The patients were asked to view nine pairs of horizontally flipped images represented on eye tracker mounted PC display (Tobii TX60, Tobii Inc., Sweden). Since a pair of flipped image has, one hand, similar consisting elements, the other hand, the right-left-reversed spatial location of the include item, we tested if the neglect symptom affect to a symmetry of the gaze distribution between a pair of image. In order to quantify an

extent of gaze shift, pixel from left end to right end was normalized as from -1 to 1, and then calculated asymmetry index (AI) as the median of eye trajectories of a pair of flipped images for the first 0.5 seconds (AI_{first}) and later 4.5 seconds (AI_{search}). The results clearly revealed that USN group showed significantly larger extent of rightward gaze shift (AI tended close to 1) as compared to RHD group. Both of AI_{first} and AI_{search} showed statistically significant correlation with BIT score, suggesting that (1) the analysis in free viewing of flipped image effectively demonstrate neglect behavior and (2) initial fixation point largely affected to later "biased" visual search. Our proposed method would be a simple and effective method for the evaluation of an extent of neglect behavior.

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Poster

608. Brain Stimulation and Recovery After Stroke

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Topic: C.08.Stroke

Title: Dissociative evaluation of neglect behavior and attention deficit based on the spatial distribution of reaction time in patients with unilateral spatial neglect

Authors: *N. KAWASHIMA[1], Y. TAKAMURA[2], S. OHMATSU[2], H. ABE[3], K. IKUNO[4], K. TANAKA[5], A. MANJI[6], T. TOMINAGA[7], S. MORIOKA[2];
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Abstract: Unilateral spatial neglect (USN) is a common neurological syndrome following predominantly right hemisphere damage. While USN is classically regarded as a parietal syndrome, it has become evident as a widespread attention network disorder. We here aimed to propose a dissociative evaluation between spatial neglect and attention deficit based on the characteristics of the spatial distribution of the reaction time. We also focused on the aspect of the ventral attention network disruption which cannot be evaluated by paper-based neuropsychological test. 144 right-brain-damaged patients participated, and were categorized into three subgroups based on the score of the behavioral inattention test (BIT): (1) USN++ (n=47) with a BIT score below the cut-off (<131); (2) USN+ (n=59) with a BIT score above the cut-off (>131) but with experience of neglect in daily life and/or a previous history of USN; (3) RHD (n=38) with a right hemisphere disorder without neglect. Trail marking test A (TMT-A)

was also evaluated. The subjects were asked to perform a touch panel-based pointing task toward circles (7 columns x 5 rows) on a PC monitor, and the reaction time to each object was recorded. In order to quantify the spatiotemporal characteristics of the reaction time, the total average of all objects (RT_{mean}) and the ratio between right and left side (L/R_{ratio}) were calculated. The result demonstrated that RT_{mean} has a strong relevance to TMT-A ($r=.56$, $p<0.01$), and L/R_{ratio} has a statistically significant correlation with BIT ($r=-.31$, $p<0.01$). Since BIT reflect not just neglect but attention deficit, there was a strong correlation between BIT and TMT-A ($r=-.73$). On the other hand, L/R_{ratio} showed weak correlation with RT_{mean} ($r=.24$), suggesting that these two parameters distinctly reflect the aspect of neglect and attention deficit, respectively. Interestingly, increase of L/R_{ratio} (prolongation of reaction time for left targets) was clearly shown in most of patients in USN+ groups, suggesting that our proposed method have potential as a sensitive probe to judge subtle neglect that cannot be detected by a paper-based neuropsychological test. These results suggest that spatial distribution of reaction time might be a useful tool not only for the evaluation of USN, but also for the dissociative evaluation between neglect and attention deficit.

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Poster

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Burroughs Wellcome

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A*STAR NSS

Title: A neural-interface to boost motor function after stroke

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Abstract: Introduction. Stroke is the leading cause of permanent and long-term disability worldwide, and it is crucial to develop strategies to improve rehabilitation. Recent work from our lab has found that perilesional low frequency (LF) oscillatory dynamics were abolished after stroke and then reinstated after motor rehabilitation. Importantly, a subset of the animals did not experience recovery and continued to have persistent deficits. This study aimed to further characterize and develop a method to therapeutically modulate LF dynamics in animals with poor recovery. Direct current stimulation (DCS) has been used to modulate ongoing neural dynamics and excitability. We thus examined the hypothesis that DCS can boost task-related LF dynamics and enhance motor function, by testing the effects of brief on-demand DCS delivered to the perilesional cortex in animals with poor recovery.
Method. Long Evans rats were trained on a skilled forelimb reach-to-grasp task. After performance stabilized, we induced a focal photothrombotic stroke in the forelimb motor cortex. We then implanted microwire arrays in the rostral perilesional cortex, and recorded local field potential (LFP) and single unit activity during motor rehabilitation to investigate the associated oscillatory dynamics. We assessed the neural effects of DCS by recording neural activity during epidural DCS under anesthesia in healthy Long Evans rats. To probe behavioural effects of DCS, we induced stroke in a separate group of rats and compared performance with DCS, no stimulation, sham and alternating current (AC) stimulation applied during motor execution (i.e. after recovery had plateaued).
Results. Recovery after stroke was associated with an increase in power, inter-trial coherence and spike-field coherence (SFC) in the LF band. DCS was effective at modulating LF dynamics, increasing LF power and SFC in anesthesized rats. Furthermore, DCS, compared to no, sham and AC stimulation, improved task performance in rats with chronic motor deficits after stroke. Importantly, these animals had achieved a plateau with low levels of success even after extensive practice and DCS increased performance beyond this plateau level.
Conclusion. Low frequency oscillations are important markers of recovery after stroke, and can be targeted to enhance motor function during rehabilitation. We demonstrate here a novel, easily-translatable stimulation paradigm to modulate ongoing neural dynamics and to improve motor performance.

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Poster

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Title: Functional and neurophysiological outcomes of intensive hand retraining in the acute phase post stroke: a pilot study.

Authors: *M. YAROSSI[1,2], J. PATEL[3], Q. QIU[3], G. FLUET[3], A. MERIANS[3], S. ADAMOVICH[2], E. TUNIK[4];
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Abstract: Upper extremity deficits can profoundly affect independence post stroke. Recovery of dexterous hand function has been found to be particularly recalcitrant to intervention, and the neurophysiological mechanisms underlying functional improvement remain unclear. Evidence from human and animal investigations suggest the first month after stroke is a critical time for synaptic plasticity in peri-infarct zone when impairment focused training may be most effective. The objective of this ongoing pilot study is to investigate the effect of focused intensive hand therapy in the acute stage post-stroke. Following informed consent 11 subjects (4 female, 61.4±15.7 years, 15.3±11.6 days post stroke) received 60 minutes of training 5 days/week for two weeks in addition to their on-going in/out-patient physical, occupational and speech therapy. Subjects were randomized to receive either traditional repetitive task practice training (RTP) or training using virtual reality gaming simulations (VR). To assess the impact of the intervention subjects underwent a battery of clinical, kinematic and neurophysiological testing prior to (PRE), immediately following (POST), and 1 month following training (RET). Both groups improved PRE to POST on the Box and Blocks Test (BBT) and Fugl-Meyer (FM) score, however the VR group showed greater improvement at RET. Transcranial magnetic stimulation was used to measure excitable cortical territory (area) for selected intrinsic (FDI, APB, ADM) and extrinsic (EDC, FDS) hand muscles. Ipsilesional hemisphere excitable area increased for the intrinsic hand muscles across sessions, with little change in extrinsic muscles. Excitable area on the contralesional hemisphere was unchanged with training for all muscles. Changes in cortical motor topography did not differ between groups. Across groups increase in excitable cortical territory from PRE to 1M was significantly correlated with improvement in BBT ($r=0.795$, $p=0.003$) and FM ($r=0.753$, $p=0.007$) for the FDI muscle. These promising preliminary data suggest intensive hand rehabilitation in the acute phase post stroke can promote functional recovery, and training using VR increases retention 1 month following intervention. Across all groups improvement in clinical and kinematic measures was associated with expansion of the lesioned hemisphere excitable cortical territory for the intrinsic hand muscles. Recruitment of a larger patient population will answer questions regarding role of VR in intensive hand rehabilitation in the acute phase post stroke, and identify the utility of excitable cortical area as a biomarker of recovery.

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Poster

609. CNS Injury and Damage: Non-TBI

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Program#/Poster#: 609.01/BB15

Topic: C.09. Brain Injury and Trauma

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Title: Significance of hyperbaric pressure on ICP drift in porcine models

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Abstract: Aim:
Intracranial pressure (ICP) monitoring has been a standard of care in neurosurgery for trauma, neoplastic, neurovascular patients. The recent advances in calculation of cerebral perfusion pressure (CPP), pressure reactivity (PRx), optimal CPP (optCPP) has been beneficial for patient care and these indices are built on multiple parameters, including the ICP. The clinical significance of ICP reading remains substantial.
Pressure drift, the deviation of pressure readings over time, is occasionally encountered clinically. One factor to consider in the interpretation of ICP may be the atmospheric pressure fluctuation. The traditional extra-ventricular drainage is exposed to atmosphere and reflects the real-time ICP with the fluid level of the CSF drained. On the other hand, some digital ICP sensors are zeroed upon insertion and absolute value of ICP are measured with respect to the atmospheric pressure upon insertion throughout its duration of implementation without reconsidering the influence of the atmospheric fluctuation. To optimize accuracy of ICP readings and its interpretation using other variables such as CPP, PRx, optCPP, we consider the influence of atmospheric pressure fluctuations and its relevance to digital ICP readings.
Methods:
Range of atmospheric pressure fluctuations is first calculated with data downloaded from public domain. We then analyzed of atmospheric pressure readings for Taipei, New York, London and Berlin. Also, we investigated the relevance of meteorological atmospheric pressure readings to ICU atmospheric readings using data from Taipei and compared to readings within ICU at Taipei Medical University Hospital demonstrated. Lastly, Porcine models were then utilized to demonstrate the correlation between hyperbaric pressure and digital ICP recordings.

Results:
The atmospheric pressure fluctuations revealed that fluctuations at Taipei, New York, London and Berlin were beyond 5mmHg within 5 days were 18%, 75%, 72%, and 69%, respectively. Also, relevance of ICU atmospheric pressure were strongly correlated with ICU atmospheric pressure, and readings revealed a 2.24±0.22 mmHg increase in ICU atmospheric pressure over the meteorological atmospheric pressure reading. Lastly, porcine model using a hyperbaric chamber revealed consistent correlation between atmospheric pressure and ICP fluctuations.
Conclusion:
Atmospheric pressure fluctuation is a significant phenonmenon. Its influence on reading of digital ICP sensors is substantial and its role in clinical decision-making process should be further validated with additional animal studies and clinical trials.

Disclosures: J.C. Wu: None. **K. Chen:** None. **Y. Chan:** None. **Y. Chiang:** None.

Poster

609. CNS Injury and Damage: Non-TBI

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 609.02/BB16

Topic: C.09. Brain Injury and Trauma

Title: Raise of plasma and brain tissue taurine and other amino acids in a model of intracranial hemorrhage in rats

Authors: *F. C. ESTRELLA[1], L. TRISTAN[1], A. DIAZ-RUIZ[1], S. MEZA-TOLEDO[2], C. RIOS[1];
[1]Neurochemistry, Natl. Inst. of Neurol. and Neurosurg., Mexico city, Mexico; [2]Exptl. chemotherapy, Natl. Politechnic Inst., Mexico city, Mexico

Abstract: Intracranial hemorrhage (ICH) is a cerebrovascular disease with a high mortality rate. Among the main consequences arising from it is the production of edema, that in turn increases cell volume. As a mechanism to preserve its volume, cell activates regulatory mechanisms, such as the eflux of osmolytes like taurine. Previous studies, have related levels of taurine to cerebrovascular diseases, due to their increasing after the establishment of an injury and their reduction after a medical treatment. A relationship between Taurine and edema and their potential use as biomarker to predict a poor outcome in patient who suffered subarachnoid hemorrhage (SHA) has been reported too. However, association between taurine levels and edema in ICH hasn´t been directly characterized yet. In the present study, we quantified the concentration of taurine and other amino acids in plasma and cerebral tissue, in an ICH model in rat. We injected collagenase type VII intrastriatally in male Wistar rat in order to reproduce an

ICH model. After the administration of the enzyme, rats developed neurological deficits (ipsilateral circling behavior, inability to tun to the non injured side and inability for walking along the beam). Animals showing no deficit were discarded. We quantified the concentrations of taurine in plasma and in the whole injured hemisphere at different times after injection: 3, 24, 48, 72 and 96 hours(hrs), while a control group of rats was administrated with saline solution. There were no changes in the plasmatic leves of taurine in any of the groups, having similar levels of concentration as the control group at all times evaluated. Other amino acids related to cerebral injury as glutamate and glutamine, showed no change in plasma when compared with the control group. In tissue, concentration of taurine showed an increased starting at 24 to 96 hrs, when compared to control group. For glutamate, same increasing pattern was observed, being statistically significant at 96 hrs when compared to control group levels. Glutamine also showed increasing levels, with statistically significant at 72 and 96 hrs. After measuring the percentage of water, we observed a slight increase in the content of water up to 48 hrs, decreasing at 72 hrs. Hematoma area was measured by microscopy at the site of collagenase administration, showing the highest levels of hemorrhage at 24 hrs and its partial re-absoption 96 hrs latter. Increase of amino acids such as taurine, glutamate and its precursor glutamine might be considered as potential biomarkers of disease.

Disclosures: F.C. Estrella: None. **L. Tristan:** None. **A. Diaz-Ruiz:** None. **S. Meza-Toledo:** None. **C. Rios:** None.

Poster

609. CNS Injury and Damage: Non-TBI

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 609.03/BB17

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant MH094835

Title: JY-515,317, an NMDA receptor modulator with glycine site partial agonist-like properties, shows therapeutic potential for the treatment of neuropathic pain.

Authors: *N. GHOREISHI-HAACK[1], E. M. COLECHIO[1], A. L. GROSS[1], J. L. PRIEBE[1], J. DUNNING[1], J. BURGDORF[2], T. M. MADSEN[1], R. A. KROES[1], M. A. KHAN[1], J. R. MOSKAL[1,2];
[1]Aptinyx Inc., Evanston, IL; [2]Northwestern Univ., Evanston, IL

Abstract: Aim of Investigation: JY-505,317 is an N-Methyl-D-aspartic acid receptor (NMDAR) modulator that acts as a functional glycine-site partial agonist. The present studies examine the

effect of JY-515,317 in multiple rat models of neuropathic pain. Methods: The analgesic effects of JY-515,317 were evaluated in the rat Bennett model (chronic sciatic nerve constriction), the Taxol model of chemotherapy-induced pain, the streptozotocin (STZ) model of diabetic neuropathy, the formalin model of persistent pain, and the tail flick model of acute pain. Potential sedative / ataxic effects were also examined in the rota-rod test. Results: A single oral dose of JY-515,317 produced a rapid-acting (1 hr post-dosing) and long-lasting (24 hrs and 1 week post-dosing) mechanical and thermal analgesia in the Bennett model (mechanical EC_{50} = 0.3 mg/kg PO; thermal EC_{50} = 0.4 mg/kg PO), and mechanical analgesia in the STZ model (EC_{50} = 0.3 mg/kg PO) and Taxol (EC_{50} = 5 mg/kg PO) models. In contrast, the gabapentin (150 mg/kg PO) positive control was not analgesic in the Taxol model, and only produced analgesic effect 1 hr but not 24 hr or 1 week post-dosing in the Bennet and STZ models. JY-515,317 also reduced flinching in the late phase of the formalin test 1 hr post dosing (EC_{50} = 0.2 mg/kg PO) to a similar degree as gabapentin (150 mg/kg PO). JY-515,317 (1 mg/kg PO) was ineffective the tail flick model of acute pain. Lastly, JY-515,317 (1-100 mg/kg PO) did not induce a sedative / ataxic effect in the Rota-rod test when measured up to 2 hr post-dosing, whereas a therapeutic dose of gabapentin (150 mg/kg PO) did produce sedative / ataxic effects at 1 hr and 2 hrs post-dosing. Conclusions: These data show that JY-515,317 has therapeutic potential as a rapid acting and long-lasting therapeutic for the treatment of a variety of neuropathic pain conditions with no sedative or ataxic effects.

Disclosures: N. Ghoreishi-Haack: A. Employment/Salary (full or part-time): Aptinyx Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx Inc. **E.M. Colechio:** A. Employment/Salary (full or part-time): Aptinyx Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx Inc. **A.L. Gross:** A. Employment/Salary (full or part-time): Aptinyx Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx Inc. **J.L. Priebe:** A. Employment/Salary (full or part-time): Aptinyx Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx Inc. **J. Dunning:** A. Employment/Salary (full or part-time): Aptinyx Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx Inc. **J. Burgdorf:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx Inc.. F. Consulting Fees (e.g., advisory boards); Aptinyx Inc. **T.M. Madsen:** A. Employment/Salary (full or part-time): Aptinyx Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx Inc. **R.A. Kroes:** A. Employment/Salary (full or part-time): Aptinyx Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx Inc. **M.A. Khan:** A. Employment/Salary (full or part-time): Aptinyx Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx Inc. **J.R. Moskal:** A. Employment/Salary (full or part-time): Aptinyx Inc.. E. Ownership Interest (stock,

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Poster

609. CNS Injury and Damage: Non-TBI

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 609.04/BB18

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant ROI HL109025 and by resources provided by the North Florida/South Georgia Veterans Health System

Title: Hypothalamic pituitary dysfunction resulting in anxiety and decreased circulating testosterone levels following traumatic brain injury in a male rodent model

Authors: *S. ADAMS[1], C. F. CONOVER[2], S. I. SVETLOV[3], K. WANG[3], P. W. DAVENPORT[3], J. F. YARROW[3];
[1]Hlth. and Exercise Sci., Pfeiffer Univ., Salisbury, NC; [2]North Florida/South Georgia Veterans Hlth. Syst., Gainesville, FL; [3]Univ. of Florida, Gainesville, FL

Abstract: Hypothalamic-pituitary dysfunction causes anxiety and lowered circulating testosterone concentrations in soldiers experiencing traumatic brain injury (TBI) from improvised explosive devices. It is unknown if increased anxiety and reduced testosterone occurs in rodents following exposure to multiple (two) dorsal-oriented blasts or a single frontal-oriented overpressurization blast (OB). PURPOSE: Determine anxiety and the circulating testosterone concentrations following a TBI resulting from a closed-head OB to the dorsal or frontal orientations of the skull. METHODS: Male Sprague-Dawley rats aged 2 months were randomized to receive one of three treatments: 1) closed-head dorsal-oriented OB injury to the skull on day 7 (psi=78.70±2.14 SEM) and day 14 (psi=77.83±2.47 SEM) (n=17), 2) closed-head frontal-oriented OB injury to the skull on day 7 (psi=38.60±1.44 SEM) (n=5), or 3) sham OB injury (balloon pop at 90 decibels) on day 7 and day 14 (n=6). Elevated plus maze (EPM) was used to assess exploratory behavior and anxiety-like behavior in open and closed arms for 300 seconds pre and post OB(s). Blood was acquired 14 days after TBI and plasma testosterone measured via ELISA. Open arm EPM times and testosterone concentrations were compared using one-way ANOVA with post-hoc Holm-Sidak measurement. RESULTS: Open arm EPM times were 97% lower after dorsal-oriented OB injury (0.71±0.63 SEM sec, p=0.004) compared to pre-injury (24.33±7.67 SEM sec), 99% lower after frontal-oriented OB injury (0.32±0.32 SEM sec, p=0.027) compared to pre-injury (30.42 ±11.10 SEM sec), while no significance difference was observed for the control group (4.05±3.37 SEM sec, p=0.394) compared to pre-

sham (10.45±5.33 SEM sec). Testosterone was 45% lower after dorsal-oriented OB injury (2.34±0.31 SEM ng/ml, p=0.029) compared to sham OB (4.28±5.33 SEM ng/ml), while no significant difference was observed for the frontal-oriented OB injury (2.71±1.70 SEM ng/ml, p=0.168). CONCLUSION: Increased anxiety seen in the dorsal and frontal oriented OB injuries following the first injury. Lower circulating testosterone observed in male rodents following multiple (two) dorsal oriented OB injuries to the skull. Single frontal oriented OB injury did not appear to reduce circulating testosterone (despite a similar magnitude of reduction), perhaps because the primary blast vector was directed toward occipital region of skull, animals received a single blast injury, or the smaller sample size. Future research evaluating the duration of the testosterone deficit and cortisol concentrations after closed-head OB injury and the functional consequences after TBI is warranted.

Disclosures: S. Adams: None. **C.F. Conover:** None. **S.I. Svetlov:** None. **K. Wang:** None. **P.W. Davenport:** None. **J.F. Yarrow:** None.

Poster

609. CNS Injury and Damage: Non-TBI

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 609.05/CC1

Topic: C.09. Brain Injury and Trauma

Title: Environmental enrichment promotes oligodendrocyte maturation and improves functional recovery in a mouse model of preterm birth

Authors: *T. FORBES[1], B. JABLONSKA[2], V. GALLO[2];
[2]Neurosci., [1]Children's Natl. Hlth. Syst., Washington, DC

Abstract: Hypoxic damage to the developing brain is a major consequence of premature birth and is associated with long-term neurological impairment and permanent neurodevelopmental disabilities. Hypoxia predisposes the developing brain to diffuse white matter injury, a debilitating condition characterized by ventriculomegaly, decreased white matter volume, and disturbances in myelination. Oligodendrocytes, the myelin-forming glia that ensheath axons in the central nervous system, have been found to be highly susceptible to hypoxia-induced oxidative stress in both humans and rodents. Recent clinical studies have demonstrated that social, family, and environmental factors contribute to improve cognitive outcomes in premature children. However, it is unknown if the environment can modulate the outcome of hypoxia-induced white matter brain injury. This study uses environmental enrichment - enhanced stimulation at multiple cognitive, sensory, social, and motor levels to facilitate recovery in the developing brain. Using a rodent model of preterm birth – perinatal chronic hypoxia - we find

that continuous enrichment of the environment implemented within a few days after hypoxic insult promotes cellular, ultrastructural, and functional white matter recovery. By enhancing the endogenous reparative response to hypoxic brain injury, we demonstrate that environmental enrichment attenuates the effects of perinatal hypoxia and is therefore a promising avenue to restore developmental white matter integrity and function after injury.

Disclosures: **T. Forbes:** None. **B. Jablonska:** None. **V. Gallo:** None.

Poster

609. CNS Injury and Damage: Non-TBI

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Program#/Poster#: 609.06/CC2

Topic: C.09. Brain Injury and Trauma

Support: JSPS KAKENHI 15H05719

JSPS KAKENHI 16K15646

Title: The effect of focal brain cooling on KCl-induced repetitive cortical spreading depression in rats

Authors: ***Y. HIRAYAMA**[1], T. INOUE[1], H. KIDA[2], K. SUGIMOTO[1], S. SHIRAO[1], H. IMOTO[1], S. NOMURA[1], M. SUZUKI[1];
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Abstract: Introduction: Cortical spreading depression (CSD) is a reversible depolarization wave that propagates across the brain surface at low velocity. In neurological disorders, CSD may play a key role as a pathophysiological mechanism in brain damage. Focal brain cooling (FBC) reduces neuronal damage and promotes recovery in models of epilepsy, cerebral ischemia, and trauma. Based on the above results, we assume that generation and propagation of CSD can be inhibited by FBC.
Methods: Male Sprague Dawley rats were anesthetized and subjected to craniotomy that was made to establish two operation areas. One area was used to induce CSD by the drop of 1M KCl. Another area was used to observe brain activities. Changes in neuronal and vascular modalities were evaluated using the multimodal recording which can record the simultaneous changes of Brain temperature (BrT), direct-current (DC) to alternating current (AC) range of electrocorticogram (ECoG), and laser speckle imaging of cerebral blood flow (CBF) in the same brain region. Our experiment was designed to identify relative differences between the cooling

(CL) and the non-cooling (NC) group (n = 7 each). We perfused warm or cold saline to control brain temperature at around 37 or 15 °C in each group, respectively. We also measured the effects of FBC on protein levels of endothelial NO synthase (eNOS) using western blot analysis. Results: In the NC group, KCl predominantly exhibited repetitive CSD events (propagation of hyperemia and depolarization and AC suppression of ECoG; mean frequency = 11.57). In the CL group, FBC increased the duration of all CSD events thereby the frequency of CSD was gradually reduced (mean frequency = 0.86). Moreover, eNOS was decreased in the cooled brain region compared with that in the NC group.
Discussion: K+ concentration increase due to the application of KCl to the brain surface causes depolarization and spreading away from a site of KCl application point. When FBC is applied, cooled neurons depolarize the membrane potential to stay of voltage gated Na+ channel inactivation and vasodilation induced by NO is impaired under the cooling condition. The results obtained show that FBC has an impact on the characterization of CSD such as depolarization and eNOS expression.

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Poster

609. CNS Injury and Damage: Non-TBI

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 609.07/CC3

Topic: C.09. Brain Injury and Trauma

Title: Water maze strategies used by C57/B16 mice exposed to radiation and pomegranate juice

Authors: *P. V. LORENZO[1], M. DULCICH[2], N. M. BAJWA[2], R. E. HARTMAN[2];
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Abstract: Our laboratory has shown that dietary supplementation with pomegranates, which have a high concentration of bioactive phytochemicals (plant-based compounds such as polyphenols), can improve memory function in humans following open heart surgery, ameliorate neuropathology and spatial learning/memory deficits in tg2576 mice, promote neurogenesis in wildtype mice, and improve rotarod performance in male (but not female) wildtype mice. We have also previously reported that exposure to 2 Gy of proton radiation induced depression-like behaviors in the tail-suspension test that were ameliorated by pomegranate supplementation. In this same study (Dulcich & Hartman, 2013), we showed that irradiation did not impair performance in the water maze, a test of spatial learning and memory. However, a number of other studies (e.g., Janus, 2004; Brody & Holtzman, 2006) have shown that mice can use several

strategies to "solve" the water maze. In the present study, we assessed search strategies from the mice in the Dulcich & Hartman (2013) paper to determine whether irradiation, diet, and/or gender affected the use of strategies to solve the water maze. Swim paths during the water maze trials were digitized and visually categorized into one of seven search strategies (spatial direct, spatial indirect, focal correct, focal incorrect, scanning, random search, and repetitive looping). A discriminate function analysis demonstrated that experimental group predicted search strategy use, correctly identifying 66% by gender, 61% by irradiation status, and 54% by diet. Furthermore, cell counts showed that females had more cells in the subgranular layer of the hippocampus, and that a higher cell count in the dentate gyrus was significantly correlated with increased use of a focal incorrect search strategy. These data demonstrate that the water maze can generate a much richer data set than simple escape latency and/or swim distance, and that different experimental groups can achieve similar performance on this test by the use of a number of different search strategies.

Disclosures: P.V. Lorenzo: None. **M. Dulcich:** None. **N.M. Bajwa:** None. **R.E. Hartman:** None.

Poster

609. CNS Injury and Damage: Non-TBI

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Program#/Poster#: 609.08/CC4

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant MH094835

Title: JY-515, 317 is a NMDA receptor modulator with glycine site partial agonist-like properties: *In vitro* and *In vivo* pharmacology

Authors: *A. L. GROSS[1], J. S. BURGDORF[2], X.-L. ZHANG[4], E. M. COLECHIO[1], N. GHOREISHI-HAACK[1], M. E. SCHMIDT[1], S. SAHU[1], P. P. KANSARA[1], E. C. RODRIGUEZ[1], E. A. POLLARD[1], T. M. MADSEN[1], P. K. STANTON[4], M. A. KHAN[1], R. A. KROES[1], J. R. MOSKAL[1,3];
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Abstract: Aim of Investigation: JY-515, 317 is an orally bioavailable small molecule NMDA receptor modulator. The present studies detail the in vitro and in vivo characteristics of JY-515, 317. Methods: Functional glycine-site agonist effects were measured using an *in vitro* [^{3}H]MK-801 binding assay in membrane extracts prepared from human NMDAR2A-2D subtype-

expressing HEK cells. NMDA current was measured in pharmacologically isolated NMDA currents in Schaffer collateral-evoked EPSCs in CA1 pyramidal neurons using whole-cell patch clamp recordings. Long-term potentiation (LTP) was measured at Schaffer collateral CA1 synapses in rat hippocampal slices following acute bath application of JY-515, 317 as well as 24 hours post-dosing *in vivo*. Learning and memory as well as behavioral effects were measured in the rat positive emotional learning, Morris water maze, and Porsolt tests. Sedative / ataxic effects were measured in the rat Rota-Rod test. Oral bioavailability was measured in rat plasma by LC-MS/MS. Results: In recombinant human NMDAR2A-2D -expressing HEK cells, partial agonist activity of JY-515, 317 was demonstrated at all 4 receptor subtypes, with greater potency at NMDAR2B (EC_{50} of 55 pM, 28 Fm, 11 pM, 55 pM and % maximal activation relative to glycine of 41%, 47%, 63%, and 58% for NMDAR2A, NMDAR2B, NMDAR2C, NMDAR2D, respectively). JY-515, 317 also increased NMDA current in hippocampal slices at concentrations between 100-500 nM, but not at 5 μM. Similarly, in the hippocampal LTP assay, JY-515, 317 facilitated LTP at concentrations between 100-500 nM, but not at 2 μM. JY-515, 317 (1 mg/kg PO) facilitated *ex vivo* hippocampal LTP 24 hrs post-dosing. JY-515, 317 (1 mg/kg PO) facilitated Morris water maze learning from 1 hr to at least 5 days post-dosing. JY-515, 317 also produced an antidepressant-like effect in the Porsolt test (EC_{50} = 6 μg/kg PO) and facilitated positive emotional learning (EC_{50} = 3 μg/kg PO) 1 hr post-dosing. JY-515, 317 (1-100 mg/kg PO) did not show sedative / ataxic effects in the Rota-Rod test when measured up to 2 hr post-dosing. JY-515, 317 showed ~50% oral bioavailability (IV vs PO plasma AUC). Conclusions: These data demonstrate that JY-515, 317 is an orally bioavailable NMDAR modulator that acts as a functional glycine-site partial agonist. JY-515, 317 facilitates LTP and NMDA current through its interaction with the NMDA receptor where it has affinity for all 4 NR2B subtypes. JY-515, 317 shows efficacy in multiple learning and memory models as well as behavioral models without sedative or ataxic effects.

Disclosures: The Disclosure Block has exceeded its maximum limit. Please call Tech support at (217) 398-1792 for more information.

Poster

609. CNS Injury and Damage: Non-TBI

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 609.09/CC5

Topic: C.09. Brain Injury and Trauma

Title: Development and characterization of a new model of posttraumatic epilepsy

Authors: *J. SZU, D. ORNELAS, S. CHATURVEDI, H. PARK, D. BINDER;
Univ. of California, Riverside, Riverside, CA

Abstract: Traumatic brain injury (TBI) is a major cause of morbidity and mortality in the United States and is a primary public health concern. Injury to the brain may result in serious behavioral and neurological deficits. A consequence of TBI is the development of posttraumatic epilepsy (PTE) where recurrent spontaneous seizures occurs after a brain injury. The pathophysiology in which trauma to the brain leads to spontaneous seizures is unknown and clinically relevant models of PTE are key to understanding the molecular and cellular mechanisms underlying the development of PTE. Current models of PTE have focused on using pentylenetetrazole (PTZ) for testing seizure susceptibility. For example, injured animals injected with a subdose of PTZ were more susceptible to generalized seizures and displayed a decrease in latency to the first spike of an epileptiform discharge as compared to control animals. Diffusion MRI have also associated hippocampal damage to decreased seizure susceptibility in animals that have undergone a TBI, however, a correlation between cortical damage and seizure susceptibility was not observed. In our study, we utilized optical coherence tomography (OCT) to detect changes in tissue dynamics in a controlled cortical impact (CCI) injury mouse model of severe TBI (sTBI). Additionally, animals underwent *in vivo* intrahippocampal electrical stimulation for the assessment of electrographic seizure threshold (EST) and electrographic seizure duration (ESD). OCT imaging revealed structural changes within the cortex after sTBI and injured animals had a lower EST compared to sham controls. Altogether, our data suggest that EST and ESD can be quantitatively determined and OCT can be a potentially powerful imaging tool for optical biomarker detection in our model of PTE.

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Poster

609. CNS Injury and Damage: Non-TBI

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Topic: C.09. Brain Injury and Trauma

Support: NIH T32GM007752

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Title: Lysophosphatidic acid as a key initiating factor in premature infantile post-hemorrhagic hydrocephalus

Authors: *N. C. STODDARD[1,2], Y. C. YUNG[1], M. MERETE[2], J. NHAN[2], J. CHUN[1];
[1]The Scripps Res. Inst., La Jolla, CA; [2]UCSD, La Jolla, CA

Abstract: Post-hemorrhagic hydrocephalus (PHH) is a common neurological disorder associated with intraventricular hemorrhage. It presents with increased intracranial pressure leading to ventriculomegaly, severe cognitive disability, and possible death. Current treatment options are limited to palliative neurosurgical interventions following development of chronic disease. Previous studies have linked the bioactive lipid lysophosphatidic acid (LPA), signaling through cognate G protein-coupled receptors (LPARs), to the initiation of PHH during embryonic life. LPA circulates within the blood, demonstrating elevated concentrations following hemorrhage or trauma. Here, we present a novel mouse model of PHH that implicates LPA signaling at a development period correlated to premature human infants, a group with high risk for hemorrhage and subsequent PHH. Neonatal mice, approximating infants born at 20-32 weeks, were injected intracranially with LPA or vehicle to simulate intraventricular hemorrhage. These injections cause early disruption of ventricular ependymal cells, resulting in dramatic loss of cilia within 24 hours. Allowing 7 days for PHH progression results in severe ventriculomegaly, continued disruption of the ependymal lining, and increased intracranial pressure. These effects can be ameliorated by LPAR knockout. Importantly, this mechanism is distinct from fetal PHH based on affected cell populations and LPAR expression patterns. Ongoing studies include characterization of chronological receptor-mediated events along with efforts to determine the efficacy of LPAR-targeted pharmacological agents as a preventative measure against PHH development.

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Poster

609. CNS Injury and Damage: Non-TBI

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Topic: C.09. Brain Injury and Trauma

Support: grant-in-aid for scientific research on innovative areas. No. 24116007

Intramural Research Grant. No. 21-9

Title: Gene expression changes in the microglia during fear memory processing

Authors: ***Z. YU**[1], H. FUKUSHIMA[2], C. ONO[3], M. SAKAI[3], Y. KASAHARA[3], T. KIKUCHI[3], Y. TAKAHASHI[4], H. MATSUOKA[4], S. KIDA[2], H. TOMITA[3];
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Abstract: Post-traumatic stress disorder (PTSD) is a complicated central nervous system (CNS) syndrome that characterized by recurrent trauma-related memories. Maintenance of excessive fear states (reconsolidation) or failed to erase fear memories (extinction) could be considered a pathology of PTSD. Animal behavioral study indicates that re-exposure to contextual fear conditioning in a short time influence reconsolidation, and prolonged re-exposure facilitated extinction. Evidence suggested that immunological mechanisms might be involved in the pathophysiology of PTSD. Murine studies showed that microglia, the major CNS immune cells that produced inflammation cytokines could influence contextual fear memory, which suggested microglia as modulator of memory formation. However microglial functions on the fear memory reconsolidation and extinction function remains poorly understood. Herein, we used microarray experiment that base on murine contextual fear extinction model, in order to investigate the activation of microglia on reconsolidation and extinction. Our results show that transmission of nerve impulse relevant genes were significantly over-represented among fear memory consolidation and reconsolidation induced genes, whereas among fear memory extinction reduced genes. M1 microglial production of inflammation cytokines was significantly decreased and anti-inflammation cytokines were significantly increased during extinction compared with fear memory reconsolidation. Our findings indicate that activated microglia may be involved in fear memory consolidation and extinction. Microglia expression neurotransmitter receptors and product inflammation cytokines as a complex interacting network that can effect on fear memory.

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Poster

609. CNS Injury and Damage: Non-TBI

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 609.12/CC8

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant MH094835

Title: JY-515, 317, a NMDA receptor modulator with glycine site partial agonist-like properties, has neuroprotective effects in a rat model of blast-induced brain injury.

Authors: *E. M. COLECHIO[1], J. S. BURGDORF[2], A. L. GROSS[3], P. K. STANTON[4], R. A. KROES[3], R. A. KROES[3], M. A. KHAN[3], C. CEARLEY[3], T. MADSEN[3], J. R. MOSKAL[3,2]; [1]Dept Neurosci, Aptinyx, Inc, Evanston, IL; [2]Biomed. Engin., Northwestern Univ., Evanston, IL; [3]Aptinyx, Inc., Evanston, IL; [4]Cell Biol. & Anat., New York Med. Col., Valhalla, NY

Abstract: Background: JY-505,317 is an N-Methyl-D-aspartic acid receptor (NMDAR) modulator that acts as a functional glycine-site partial agonist. In recombinant human NMDAR2A-2D -expressing HEK cells, partial agonist activity of JY-515, 317 was demonstrated at all 4 receptor subtypes, with the greatest potency at NMDAR2B. JY-515, 317 also enhanced whole cell NMDA current in CA1 pyramidal neurons in hippocampal slices at concentrations of 100-500 nM but not at 5 μM. Similarly, JY-515,317 enhanced the magnitude of long term potentiation (LTP) at Schaffer collateral-CA1 synapses in rat hippocampal slices at concentrations of 100- 500 nM but not 2 μM. JY-515, 317 (1 mg/kg p.o.) also facilitated *ex vivo* hippocampal LTP and significantly increased the number of mature dendritic spine morphologies 24 hrs post-dosing. JY-515, 317 is an orally available compound that has also shown efficacy after PO administration in several animal models of learning and memory without demonstrating side effects. JY-515, 317 has approximately 50% oral bioavailability in rats (i.v. vs p.o. plasma AUC). JY-515, 317 (1 mg/kg p.o.) facilitated Morris water maze learning from 1 hr to at least 5 days post-dosing. JY-515, 317 (1-100 mg/kg p.o.) did not show sedative/ataxic effects in the Rota-Rod test.
Methods: Blast-induced brain injury was caused following the protocol of Goldstein modified for use in rats (Goldstein et al., 2012). Briefly, an aluminum shock tube (183 x 61 cm; L-3 Applied Technologies, USA) was positioned 10 cm from the head of the rat. Rats received a single ~45 PSI blast of helium generated by puncturing 0.014 inches of polyester film. Sham controls were placed outside of the blast radius. Animals were dosed with JY-515, 317 (1 mg/kg PO) or 0.5% Na-CMC in 0.9% sterile saline vehicle (1 ml/kg p.o.) 1, 2, 6, or 24 hrs post-blast. Latency to recover from anesthesia was recorded. Recovery was defined as displaying eyeblink and righting reflexes, and normal ambulation. Rats were tested in a learning task 48 hrs post-blast to evaluate potential neuroprotective effects. In addition, changes in tau phosphorylation were assessed immunohistochemically in the hippocampal formation.
Results: A neuroprotective effect was observed in rats treated with JY-515, 317(1 mg/kg, p.o.) up to 24 hours post-blast. In rats dosed 1 hour post-blast, JY-515, 317 produced a neuroprotective effect between 0.1-10 mg/kg with an EC50 = 0.2 mg/kg. Increases in phospho-tau (pSer202 + pThr205) staining were observed in the hippocampal formation in vehicle-treated controls whereas no increase in staining was observed in the JY-515, 317 -treated rats.

Disclosures: E.M. Colechio: A. Employment/Salary (full or part-time): Aptinyx, Inc. **J.S. Burgdorf:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx, Inc.. F. Consulting Fees (e.g., advisory boards); Aptinyx, Inc. **A.L. Gross:** A. Employment/Salary (full or part-time): Aptinyx,

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Poster

609. CNS Injury and Damage: Non-TBI

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 609.13/CC9

Topic: C.09. Brain Injury and Trauma

Support: DHA HU0001-15-2-0020

Title: Transient disruption of home cage activities and assessment of orexin immunoreactivity following concussive or blast-induced brain injury

Authors: *P. A. VU[1], L. B. TUCKER[2,3], J. LIU[2], E. MCNAMARA[1], A. H. FU[2,3], Y. KIM[2,3], J. T. MCCABE[2,3];
[1]Grad. Program in Neurosci., [2]Dept. of Anatomy, Physiology, and Genet., Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD; [3]Ctr. for Neurosci. and Regenerative Med., Bethesda, MD

Abstract: Traumatic brain injury (TBI) has become recognized as an invisible wound of modern warfare. The employment of improvised explosive devices by enemy combatants exposes our warfighters to both primary blast wave-induced brain injury as well as secondary concussive

injuries. The majority of reported TBI cases from recent conflicts are mild with sleep-wake disturbances being among the most common clinical complaints. The objective of the current study was to assess the acute effects of concussive brain injury (CBI) and blast wave-induced brain injury, separately. Male C57BL/6J mice (approximately 10 weeks old) were randomly assigned to receive CBI, which was delivered via a Leica ImpactOne controlled cortical impact device; be exposed to blast overpressure of approximately 15 psi in the Advanced Blast Simulator (ABS); or undergo sham procedures for each injury model. Injured animals and their respective shams were further divided into the following subgroups: 24-hour survival in standard housing, 72-hour survival in standard housing, and 72-hour survival in Any-Maze cages (Stoelting, Co.). The Any-Maze cages were used to continuously monitor home cage activity. Mice from each group were tested in an open field, and on the y-maze test for spontaneous alternation behavior (working memory) before brain tissue was collected for analysis of immunoreactive orexin-A neurons. This group of hypothalamic neurons was previously implicated in sleep-wake disorders. Preliminary results showed that both CBI and ABS caused significant, but transient decreases in home cage activities, including wheel running and ingestive behaviors 24 hours postinjury. Furthermore, ABS resulted in general hypoactivity in a 20-minute open field session at both time points for standard-housing animals, but not for Any-Cage-housed animals. This decrease in activity was accompanied by a reduction in orexin-A hypothalamic neurons. In contrast, CBI did not result in hypoactivity in the open field nor did it cause a reduction in orexin-A positive neurons. Instead, mice subjected to CBI spent more time in the center of the open field with no difference in the overall distance travelled during a 20-minute open field session relative to their sham controls. The increase in activity in the center of the open field suggested that CBI mice demonstrated less anxiety than their sham controls. The differential effects of CBI and ABS may speak to the heterogeneous presentation of patients suffering from mild TBI, who often report no or very minor symptoms.

Disclosures: **P.A. Vu:** None. **L.B. Tucker:** None. **J. Liu:** None. **E. McNamara:** None. **A.H. Fu:** None. **Y. Kim:** None. **J.T. McCabe:** None.

Poster

609. CNS Injury and Damage: Non-TBI

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 609.14/CC10

Topic: C.09. Brain Injury and Trauma

Title: Quantitative and qualitative assessment of glymphatic flux using Evans blue albumin in rats

Authors: M. WOLF[1], Y. CHEN[1], D. SIMON[1], M. MANOLE[1], L. A. NEW[1], H. ALEXANDER[1], P. M. KOCHANEK[1], *R. S. CLARK[2];
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Abstract: Introduction: The glymphatic system is a dynamic pathway for clearance of protein and other macromolecules from brain. Normal variation in glymphatic flux occurs under physiologic conditions, e.g. sleep, and recently inefficient or overwhelmed glymphatic flux has been implicated in neurological disease, e.g. amyloid- and tau-opathies. We capitalized on the colorimetric and fluorescent properties of Evans blue labeled albumin in order to evaluate glymphatic flux in rodent models of neurological disease. Methods: 25 µl of 1% Evans blue albumin was injected into the CSF of anesthetized postnatal day (PND) 17 rats via intracisternal (i.c.) injection. This concentration results in complete binding of Evans blue to albumin, thus movement of albumin rather than unbound Evans blue can be traced. Serum was collected and brains harvested at several time points after i.c. injection. Brains were immersed in 3 ml 10% formamide for 3d to extract Evans blue. Evans blue was quantified spectrophotometrically (620nm) against a standard curve. Transit of Evans blue from CSF to blood was monitored qualitatively using intravital multiphoton microscopy and multilabel immunohistochemistry (excitation 530-550nm/emission 590nm). Results: The concentration of Evans blue albumin in serum was 2.1±0.9, 4.3±0.1, 2.0±0.3, and 0.4±0.2 µg/ml; and in brain was 12.2±2.2, 13.3±3.7, 9.2±1.7, and 4.5±1.1 µg/ml at 1, 4, 24, and 72h, respectively (n=4/group). Peri-arteriolar Evans blue albumin was detected using intravital multiphoton imaging at 1h after CSF injection in live PND17 rats. Sagittal sections from rats 2h after i.c. injection showed extravasation of Evans blue albumin from ventricles and leptomeningeal spaces. This extravasation appeared markedly increased when intracranial pressure was increased to 40mmHg in the rats. Conclusion: Injection of Evans blue albumin can be used to dynamically assess protein flux from CSF to brain to blood. This technique may be useful for the study of glymphatic flux in both physiologic and pathophysiologic conditions, including elevated intracranial pressure.

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Poster

609. CNS Injury and Damage: Non-TBI

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 609.15/CC11

Topic: C.09. Brain Injury and Trauma

Support: NIH R21 HD080573-02

Department of Anesthesiology

Title: Multiple neonatal anesthetic exposures has more severe effects on adult rodent behavior

Authors: *R. MAKARYUS[1], J. ROBINSON[2], A. JAN[2], T. FLETCHER[2], G. ENIKOLOPOV[1], H. BENVENISTE[1];
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Abstract: Background: Anesthesia administration during neuronal development results in an increase in the number of neurons undergoing apoptosis in the mammalian brain. We previously used proton magnetic resonance spectroscopy (^{1}HMRS) to demonstrate that growth of N-acetyl-aspartate (NAA), a marker for neurons, is more severely impaired with multiple anesthesia exposures than with a single exposure, even when total anesthesia duration is the same. Given this, we hypothesized that multiple neonatal exposures would cause a more severe effect on behavior compared to a single neonatal exposure.
Methods: On post-natal day (PND) 5, male rat pups were divided into three groups designated as 'single exposure', 'multiple exposure', and 'unexposed'. For the single exposure group, the pups were exposed to 6 hours of sevoflurane anesthesia on PND 7 only; for the multiple exposure group, the pups were exposed to 2 hours of sevoflurane anesthesia each on PND 5, 7, and 10; while the unexposed group received no anesthesia. The pups were then assessed through a series of behavioral tests after PND 35, specifically by open field and novel object recognition (NOR) testing.
Results: We had 10 rats per group for behavior testing, with a total of 30 rats. Open field testing demonstrated multiple significant differences between the groups, with the multiple exposure group showing the greatest deviation from normal, see Fig. 1. Results of NOR testing is seen in Fig. 2, indicating a trend for exposed animals to spend less time with the novel object, with the multiple exposure group spending the least time with the novel object, however, not statistically significant.
Discussion: ^{1}HMRS data from previous work indicated that brain maturation, as measured by NAA, is more severely impeded, with longer lasting effects, by multiple exposures than by single exposure. Here we have demonstrated that multiple exposures to anesthesia also have a more profound effect on animal behavior than a single anesthesia exposure, even though the total exposure duration was the same for both groups.

Figure 1

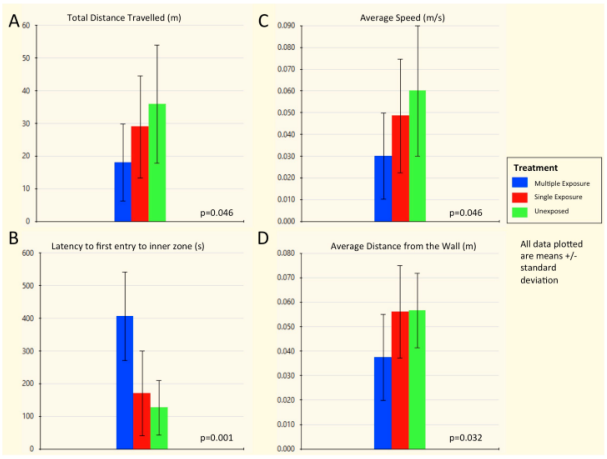

Figure 1: *Results of the open field test demonstrate that animals in the multiple exposure group had a far greater deviation from the unexposed than the single exposure group. This is seen in (A) distance travelled during testing, which also correlates with (B) the average speed of the animals per group. (C) The latency to first entry to the inner zone was highest in the multiple exposure group, possibly indicating a higher level of anxiety. (D) Average distance from the wall, with lower numbers interpreted as an increase in thigmotaxis, was lowest among those who had the multiple anesthesia regimen, again indicating possible*

Figure 2

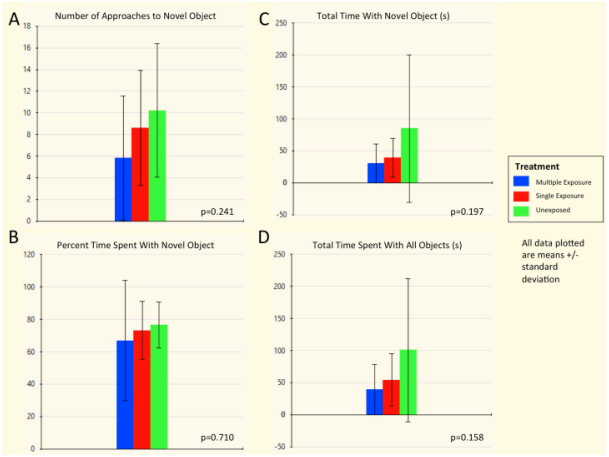

Figure 2: *Results of the NOR test are shown here. Though there are no significant differences among the three groups as determined by ANOVA, there are still important trends to note. First, demonstrated in (A) the number of approaches to the novel object, or interest in the novel object tended to be greater in unexposed group, as well as (B) the total time with the novel object. (C) The amount of time spent with the novel object as a percentage of total time with all objects also had an interesting trend. (D) Total time with both objects is also depicted here, with the multiple exposed group showing the least interest in interacting*

Disclosures: R. Makaryus: None. **J. Robinson:** None. **A. Jan:** None. **T. Fletcher:** None. **G. Enikolopov:** None. **H. Benveniste:** None.

Poster

609. CNS Injury and Damage: Non-TBI

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 609.16/DD1

Topic: C.09. Brain Injury and Trauma

Title: Delayed onset spinal epidural hematoma with development of Brown-Séquard syndrome

Authors: *S. D. ALBANO, B. W. BERMAN;
Neurosurg., Desert Regional Med. Ctr., Palm Springs, CA

Abstract: Background:
Spinal epidural hematoma makes up a minority of spinal injuries. The disease process also represents a challenge in diagnosis when not apparent on initial computerized tomography (CT) scan as the mechanism of injury and force of impact do not directly correlate with incidence of spinal epidural hematomas. Further adding to the diagnostic challenge is the fact that posttraumatic spinal epidural hematomas are rarely associated with fractures[2].
Case Report:
A 74 year old female with a history of transient ischemic attack on Plavix and aspirin 81mg daily, presented for non-radiating upper back pain after a fall from approximately four foot height. No preceding weakness, headache, lightheadedness. Post fall, no loss of consciousness, blurry vision, tinnitus, post nasal drip, otorrhea, loss of bowel or bladder control, nausea, vomiting, motor weakness or paresthesias. However in hospital patient developed episodes of vomiting. Initial physical exam demonstrated 5/5 motor strength and sensation to purposeful touch intact in upper and lower extremities. Initial CT scans showed no intracranial hemorrhage or skull fractures. Cervical spine demonstrated multiple osteophytes from cervical 4-cervical 7. Patient progressed to develop urinary retention and motor deficits. MRI demonstrated spinal epidural hematoma. Patient was taken for emergent decompressive laminectomy. Post operatively patient exhibited Brown-Séquard Syndrome. Motor deficits improved with physical therapy.
Conclusions:
Many options exist for diagnosis of spinal epidural hematoma to include CT scan, myelogram, and MRI. As demonstrated in this case spinal epidural hematoma does not need to be associated with a fracture of the vertebra and can be occult on CT scan. Therefore in the setting of progressive neurologic decline an MRI is indicated and can better elucidate disease process. Treatment options include high dose steroids and observation. However the standard of care in progressive neurologic decline is still emergent decompression.

Disclosures: S.D. Albano: None. **B.W. Berman:** None.

Poster

610. Cellular and Sub-Cellular Imaging: Markers of Stress, Trauma, and Stroke

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 610.01/DD2

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: IBS-R015-D1

Title: *In vivo* multi-photon imaging of cerebrovascular changes in chronically stressed brain

Authors: ***S. LEE**[1], J. MIN[2], B.-M. KANG[2], J. KIM[1], M. SUH[1,2];
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Abstract: Repeated stress exposure can increase neurotoxic signals leading to neural dysfunction alongside with alterations of neurotransmitter and neuromodulator release in the synapse. These misregulated environments can cause dysfunction of neurovascular coupling units. We previously reported that 3-week chronic stress decreased hemodynamic response and vascular reactivity and further induced changes in neuronal population and vasomodulatory system in rat restraint stress model. In the present study, we investigated chronic stress effects on the cerebrovascular structure in the mice brain cortex using *in vivo* two-photon imaging method. Two-photon (2p) microscopy provides 3-D vascular structural images in the deep tissue of live animal. Utilizing a chronic cranial window, we could trace the longitudinal vascular structural changes under chronic stress in the same animal. For *in vivo* 2p imaging of the brain, the mice had a cranial window surgery 4-6 weeks prior of imaging session. Before stress protocol, the baseline vascular image was obtained by intravenous injection of texas-red conjugated dextran. After 3-week immobilization stress (6hr/day) session, we found that chronic stressed group showed alterations in cerebral vasculature compared to control group. In stressed group, there was a reduction in both number and volume of larger vessels than capillaries. Also, stressed animal exhibited weakened blood brain barrier integrity, resulting in increased extravasation of fluorescent dye. These results suggest chronic stress may affect cerebrovascular system and their structural solidity. These effects eventually elevate the risk of neurovascular dysfunction.

Disclosures: **S. Lee:** None. **J. Min:** None. **B. Kang:** None. **J. Kim:** None. **M. Suh:** None.

Poster

610. Cellular and Sub-Cellular Imaging: Markers of Stress, Trauma, and Stroke

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 610.02/DD3

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: IBS-R015-D1

Title: Effects of chronic stress on neuronal response and connection in the sensorimotor cortex of mice

Authors: *E. BAEG[1], S. BAE[1,2], H. LIM[1,2], J. MIN[1,3], H. KIM[1,3], M. SUH[1,3];
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Abstract: We investigated the effect of chronic stress on neuronal activation in the sensorimotor cortex of mice, an important top-down circuit in attentional and sensory processes. Three weeks of physical immobilization was used to make stress animal model. Membrane voltage changes and blood volume changes from one hemisphere upon the whisker deflection were measured using voltage sensitive dye imaging (VSDI) and optical intrinsic signal (OIS) imaging techniques. Changes of voltage to whisker stimulation started from the barrel cortex and expanded to the motor cortex in normal animals because of the innate connection between sensory and motor cortices. However, the extent of membrane change in stress mouse was smaller, not just in amplitude but in the size of conducted area, suggesting compromised sensorimotor network. Supportive anatomical data for the disruptive connection between the barrel and motor cortices was obtained from immunohistochemical staining using microtubule-associated protein 2 (MAP-2), a neuron-specific cytoskeletal protein. We found the reduction of MAP-2 stained processes in the barrel cortex of stressed animals. In accordance with VSDI results, blood volume changes to whisker deflection were tended to decrease in awake stress mouse. Local field potentials that were recorded in the barrel cortex showed smaller oscillating power in gamma range (30-80hz), compared to control animals. Reduction in the number of parvalbumin (PV) interneurons, which is redeemed as the important neuronal substrate in generating gamma oscillation in the barrel cortex, was observed in stressed animals. Considering the function of gamma oscillation in attention and synchronization between cortices, the reduced gamma power can explain the attentional sensory integrative problem under chronic stress. Our findings reveal the systematic effect of chronic stress on sensorimotor circuit, including cellular death and damaged connectivity between barrel and motor cortices, and explain the neural substrate of stress induced attentional problems.

Disclosures: **E. Baeg:** None. **S. Bae:** None. **H. Lim:** None. **J. Min:** None. **H. Kim:** None. **M. Suh:** None.

Poster

610. Cellular and Sub-Cellular Imaging: Markers of Stress, Trauma, and Stroke

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 610.03/DD4

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Chronic stress alters neurovascular coupling in the rodent somatosensory cortex

Authors: *K.-Y. HAN[1,3], J. HAHN[2], D. KIM[2,4], S. LEE[2], J. MIN[2,4], B.-M. KANG[2,4], M. SUH[2,3,4];
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Abstract: The regulation of microcirculation in the brain via neurovascular coupling is crucial for meeting the metabolic demands of neurons and glia. A complex interplay of excitatory and inhibitory neurons, and astrocytes is thought to control neurovascular coupling. Emerging evidence indicates, in pathological conditions, disruptions of this tight interaction leads to the failure of adequate delivery of oxygen and glucose, accelerating disease progression. However, the exact sequence of events that leads to abnormal neurovascular coupling under pathological conditions remains poorly understood. Here, utilizing acute brain slices, which allow better control of complex signaling from neurons and glia, we investigated the interactions of neurons, glia and blood vessels in somatosensory cortex in chronic stress paradigm. To induce chronic stress, 8-weeks-old male C57BL/6 mice were immobilized for 6 hours per day for 3 weeks. The effect of restraint stress on anxiety-related behavior was assessed using the elevated plus maze before all experiments. In our anatomical experiments, we observed an increase in the number of small diameter vessels (< 9 μm in luminal diameter) in chronic stress group compared to control group. In addition, in chronic stress, the number of Parvalbumin(PV)-expressing interneurons as well as Thy1-positive pyramidal neurons decreased, while the number of reactive astrocytes increased. Our functional imaging of arterioles (5 - 10 μm in luminal diameter) with focal electrical stimulation showed both vasodilation and vasoconstriction with a small set of no response in both groups. However, vasoconstriction was more prevalent in chronic stress compared with controls (25% vs 62.5%) and changes in vessel diameter were greater (8.1 ± 0.9% vs 20.6 ± 2.1%). Vasodilation was observed more often in control (62.5% vs 25%), while the degree of dilation was similar in two groups (11.4 ± 1.5% vs 12.7 ± 2.2%). In contrast to focal electrical stimulation, NMDA stimulation induced only vasodilation in arterioles from control and chronically stressed animals with no difference in degree of dilation (14.0 ± 1.2% vs 15.9 ± 4.3%). These results show that the vascular response to neural activity is not uniform, but chronic stress shifts vessel responses toward constriction. Together, our results indicate that

chronic stress remodels the interaction between excitatory and inhibitory neurons accompanying vascular structure, which may be a key step in the development of stress-induced mental disorders and cerebrovascular diseases.

Disclosures: **K. Han:** None. **J. Hahn:** None. **D. Kim:** None. **S. Lee:** None. **J. Min:** None. **B. Kang:** None. **M. Suh:** None.

Poster

610. Cellular and Sub-Cellular Imaging: Markers of Stress, Trauma, and Stroke

Location: Halls B-H

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Program#/Poster#: 610.04/DD5

Topic: C.09. Brain Injury and Trauma

Support: Project no. LQ1605 from the National Program of Sustainability II (MEYS CR)

FNUSA-ICRC no. CZ.1.05/1.1.00/02.0123 (OP VaVpI)

Title: Real time *In vitro* imaging of axonal injury

Authors: *V. LACOVICH, V. POZO DEVOTO, M. ČARNA, G. STOKIN;
Intl. Clin. Res. Ctr. FNUSA-ICRC, Brno, Czech Republic

Abstract: Over the past 30 years, research has linked moderate and severe traumatic brain injury (TBI) to a greater risk of developing Alzheimer`s disease (AD) or other types of dementia years after the original head injury. TBI is a focal injury that results in cerebral contusions at specific location or diffuse axonal injury over a widespread area, probably due to shear forces. Intriguingly, axonal transport, including microtubule tracks, is subject to injury also during aging and in several diseases such as AD, however, mechanisms linking TBI to neurodegeneration, and vice versa, remain to be elucidated. To advance our understanding of the mechanisms underling TBI we have designed an *in vitro* system that allows real-time assessment of axonal response to injury. More specifically, we have developed a system that consists of a specifically designed microfluidic chamber coupled to an electronically controlled syringe pump and a confocal mycroscope. Briefly, human neurons were first differentiated from neural stem cells and seeded in the proximal chamber with their axons elongating through a series of parallel channels ending in the distal chamber. Perpendicularly to the axonal grid an additional channel goes from side to side of the chamber. This channel is connected to a syringe controlled by an electronical device, which sets the pressure of the syringe and ultimately controls the flow through the channel. This setting is mounted on the microscope stage and allows real time visualization of axonal transport by taking advantage of fluorescently labeled surrogate markers of axonal transport. To generate

acute shear stress on the axon we fine-tuned the applied force and duration of the flow. We first examined axonal structural markers for the extent of damage caused by different flow forces. We next evaluated transport dynamics of APP vesicles, mitochondria and lysosomes before, during and after injury. To this end we analysed proportions of anterograde, retrograde stationary movement, mean velocities, direction of reversions and density of all the particles. Changes in the dynamics immediately after the injury were observed and point toward specific mechanisms involved in TBI.

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Poster

610. Cellular and Sub-Cellular Imaging: Markers of Stress, Trauma, and Stroke

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 610.05/DD6

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant NS083858 (S.A.K.)

The Academy of Finland, FGSN, CIMO and University of Helsinki (L.K.)

Title: Reversible disruption of neuronal mitochondria by ischemic and traumatic injury revealed by quantitative two-photon imaging in the neocortex of anesthetized mice

Authors: M. KISLIN[1], J. SWORD[2], D. CROOM[2], E. PRYAZHNIKOV[1], E. LIHAVAINEN[3], D. TOPTUNOV[4], H. RAUVALA[1], A. S. RIBEIRO[3], *S. A. KIROV[2], L. KHIROUG[1];
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Abstract: Mitochondria play a variety of functional roles in cortical neurons, from metabolic support and neuroprotection to the release of cytokines that trigger apoptosis. In dendrites, the structure of mitochondria is closely linked to their function, and fragmentation of the normally elongated mitochondria indicates loss of their function under such pathological conditions as stroke and brain trauma. Using in vivo two-photon microscopy in mouse brain, we quantified mitochondrial fragmentation (fission) in a full spectrum of cortical injuries ranging from severe to mild. Severe global ischemic injury was induced by bilateral common carotid artery occlusion, while severe focal stroke injury was induced by Rose Bengal Photosensitization. A moderate and mild traumatic injury was inflicted by focal laser lesion and by mild photo-damage, respectively. Dendritic and mitochondrial structural changes were tracked longitudinally using transgenic

mice expressing fluorescent proteins localized either in cytosol or in mitochondrial matrix. In response to severe injury, mitochondrial fragmentation developed in parallel with dendritic damage signified by dendritic beading. Unlike dendritic beading, mitochondrial fragmentation spread beyond the injury core in focal stroke and focal laser lesion models. In moderate and mild injury, mitochondrial fragmentation was reversible with full recovery of structural integrity after 1-2 weeks. The transient mitochondrial fragmentation observed in the mild photo-damage model was associated with an increase in dendritic spine turnover without any signs of dendritic damage. Our findings indicate that alterations in neuronal mitochondria structure can be reversible in ischemic and traumatic injuries and point to mitochondrial fragmentation as an early sensitive indicator of neuronal damage.

Disclosures: **M. Kislin:** None. **J. Sword:** None. **D. Croom:** None. **E. Pryazhnikov:** None. **E. Lihavainen:** None. **D. Toptunov:** None. **H. Rauvala:** None. **A.S. Ribeiro:** None. **S.A. Kirov:** None. **L. Khiroug:** None.

Poster

610. Cellular and Sub-Cellular Imaging: Markers of Stress, Trauma, and Stroke

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 610.06/DD7

Topic: C.08.Stroke

Support: NIH grants NS088413

NIH grants NS085568

NIH grants NS091585

Title: Diminished astrocytic gliosis following focal cerebral ischemia in GPR37 knockout mice

Authors: ***M. Q. JIANG**[1], M. M. GIDDENS[2], X. GU[1], L. WEI[3], R. A. HALL[2], S. P. YU[3]; [1]Neurosci., [2]Pharmacol., [3]Anesthesiol., Emory Univ., Atlanta, GA

Abstract: Ischemic stroke is the third most prevalent cause of death and the leading cause of long term disability with direct and indirect costs amounting to 36.5 billion annually in the United States. Following acute cell death resulting from excitotoxic conditions, secondary damage can occur to surrounding tissues in the peri-infarct region. We have demonstrated that in wild type (WT) mice, ischemia initiates astrocytic gliosis within 6 hours following stroke. Glial fibrillary acidic protein (GFAP) positive astrocytes exhibit pronounced hypertrophy in the peri-infarct region visible within 3 days following injury. Astrocyte mediated gliosis is known to be an acute regulated process that initially aids in the formation of a barrier around brain injury to

limit the spread of noxious chemokines, excitotoxic and inflammatory factors. A mature glial scar comprising of astrocytes can be observed within 7 days following stroke. Since the G protein-coupled receptor GPR37 has recently been reported to mediate neuroprotective and glioprotective effects, we examined the role of GPR37 in ischemic brain damage by evaluating astrocytes and the development of gliosis after cerebral ischemia in WT vs. GPR37 knockout (KO) mice. Adult WT and GPR37KO mice were subjected to a focal cerebral ischemia affecting the right sensorimotor cortex. Mice were sacrificed 3 days following ischemia and histological examinations were performed on frozen 10 μm thick sections. Histological analysis of cell death was quantified using terminal deoxyribonucleotidyl transferase-mediated dUTP nick end labeling (TUNEL). Astrocytes were stained using GFAP and cell nuclei were labeled using a Hoechst dye. Robust reactive astroglia activation and scar formation around the infarct area were observed in WT animals. Reactive astrocytes accumulation was seen at 6 hours following ischemia and the number of astrocyte processes increased significantly by 7 days. A glial scar border marked by GFAP staining appeared by 48 hours following ischemia. In contrast, a glial scar failed to form after the ischemic insult in GPR37KO mice. The number of astrocytes present in the peri-infarct region was significantly reduced by about 50% compared to WT mice (n=3, p=0.0106). Consistently, the mean GFAP fluorescence intensity was significantly weaker in the peri-infarct region of GPR37 KO animals (0.41 ± 0.16 n=5) compared with matched WT mice (2.158 ± 0.3, n=4). These preliminary data suggest that GPR37 protein plays a critical role in astrocyte activation and the formation of glia scar following ischemic insults to the brain.

Disclosures: **M.Q. Jiang:** None. **M.M. Giddens:** None. **X. Gu:** None. **L. Wei:** None. **R.A. Hall:** None. **S.P. Yu:** None.

Poster

610. Cellular and Sub-Cellular Imaging: Markers of Stress, Trauma, and Stroke

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 610.07/DD8

Topic: C.09. Brain Injury and Trauma

Support: Synergy

Title: Self-renewal and differentiation capacity of cerebral cortex reactive astrocytes *In vivo*

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Abstract: Cells akin to astrocytes act as adult neural stem cells (aNSCs) in specific neurogenic niches of the adult mammalian brain and are characterized by self-renewal and generation of differentiated progeny. While *in vivo* these cells self-renew for a limited number of cell divisions and are bilineage, generating neurons and astrocytes (Calzolari *et al*., 2015; Encinas *et al*., 2011), in the neurosphere assay *in vitro* they self-renew for a long time and are multipotent, giving rise to neurons, astrocytes and oligodendrocytes. Astrocytes outside the neurogenic niches do not proliferate or generate neurons under physiological conditions, but invasive injury triggers both proliferation *in vivo* and activation of NSC potential *in vitro* in a subset of reactive astrocytes (RAs) in the cerebral cortex gray matter (GM) (Buffo *et al*., 2008; Sirko *et al*., 2013). Of note, while *in vivo* RAs undergo only a single cell division, generating two astrocytes (Bardehle *et al.*, 2013), a subset of RAs shows long-term self-renewal and multipotency *in vitro*. The aims of this study were to assess i) whether and to which extent RAs could be stimulated to proliferate several times *in vivo*; and ii) whether cortical RAs could generate different cell types when relocated to a neurogenic environment, such as an aNSC niche. Therefore, we analyzed the proliferative behavior of RAs through their response to repetitive pathological stimuli in the adult murine cerebral cortex GM. To follow cycling cells in the injured GM at different time points after stab wound injury, we used a dual-labeling method by combining two thymidine analogues, 5-bromo-2'-deoxyuridine (BrdU) and 5-ethynyl-2'-deoxyuridine (EdU). Quantitative analysis of *in vivo*-labeled cycling cells showed that repetitive pathological stimuli induced marked changes in the proliferative behavior of RAs and other glial cells (NG2-glia and microglia), and led a subset of RAs to reenter the cell cycle. To analyze the differentiation capacity of RAs *in vivo* we transplanted cortical RAs or aNSCs from the subependymal zone of adult actin-GFP mice cultured as neurospheres heterotopically into the adult dentate gyrus and the embryonic brain. Taken together, RAs can show a larger proliferative potential *in vivo* while their progeny remains more restricted compared to aNSCs.

Disclosures: **L.L. Canhos:** None. **S. Falk:** None. **M. Götz:** None. **S. Sirko:** None.

Poster

610. Cellular and Sub-Cellular Imaging: Markers of Stress, Trauma, and Stroke

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 610.08/DD9

Topic: C.09. Brain Injury and Trauma

Title: Short-duration overpressure induces astrocyte reactivity

Authors: ***N. HLAVAC**, S. MILLER, P. VANDEVORD;
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Abstract: Blast-induced neurotrauma is a complex, multisymptomatic condition that affects both military personnel and civilians. Exposure to primary blast induces acute cellular changes in the brain that precede chronic deficits in memory and behavior. Glial reactivity, particularly in astrocytes, is a prominent hallmark of acute injury. Astrocyte reactivity is primarily characterized by increased intermediate filament protein expression and hypertrophy. While evidence exists to suggest that glial reactivity is both protective and disruptive in the injured brain environment, definitive mechanistic links between acute cellular responses and chronic deficits remain to be elucidated. Moreover, the secondary injury mechanisms of blast neurotrauma that drive astrocyte reactivity are not well defined. This study aimed to characterize acute cellular response to short-duration overpressure through the use of *in vitro* models. Both astrocyte-rich cultures and mixed cell cultures with neurons and astrocytes were prepared from cortices of Sprague-Dawley rat pups. Cells were exposed to short-duration overpressure using a custom *in vitro* shock wave generator. At 24 hours, viability of astrocytes was assessed using an MTT assay. Furthermore, alterations in expression of structural proteins were analyzed using reverse transcription real-time polymerase chain reaction and Western blotting techniques. Specific targets of interest were cytoskeletal proteins, including actin, vinculin and intermediate filaments, as well as membrane proteins necessary for cell-cell communication. Astrocytes were viable at 24 hours post-exposure to overpressure. Results indicated that astrocytes in the presence of neurons become reactive by the 48 hour time point as assessed by significant upregulation of glial fibrillary acidic protein (GFAP) compared to a sham group (p-value<0.05). Furthermore, astrocyte-rich cultures also displayed significantly higher GFAP protein levels at 48 hours post-exposure compared to sham. These results imply that astrocytes can become activated by mechanical stimulus alone. Transient alterations in gene expression for ezrin and connexin-43 occurred at 24 hours post-exposure, suggesting potential avenues for secondary injury mechanisms that affect cell-cell communication and astrocyte function in the acute stages of injury. The results of this study provide evidence of structural alterations in glia as a result of short-duration overpressure exposure as well as implications for further investigations into secondary injury mechanisms involved in blast neurotrauma.

Disclosures: **N. Hlavac:** None. **S. Miller:** None. **P. VandeVord:** None.

Poster

610. Cellular and Sub-Cellular Imaging: Markers of Stress, Trauma, and Stroke

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 610.09/DD10

Topic: C.09. Brain Injury and Trauma

Support: Intramural research

RO1NS070024

Title: Blast shockwaves propagate Ca^{+2} activity via purinergic astrocyte networks in human central nervous system cells

Authors: R. RAVIN, 20892[1], P. BLANK, 20892[1], B. BUSSE[1], N. RAVIN, 20892[1], S. VIRA, 20892[1], L. BEZRUKOV, 20892[1], H. WATERS, 20892[1], H. GUERRERO-CAZARES[2], A. QUINONES-HINOJOSA[2], P. LEE, 20892[1], *R. D. FIELDS[3], S. BEZRUKOV, 20892[1], J. ZIMMERBERG, 20892[1];
[1]NICHD, Bethesda, MD; [2]Johns Hopkins Univ., Baltimore, MD; [3]NICHD, NIH, Bethesda, MD

Abstract: Blast-induced traumatic brain injury (bTBI) is a worldwide health problem. Even those experiencing low-level blast explosions can develop neurocognitive symptoms without evidence of neurotrauma, but the cellular mechanisms of this phenomenon are unknown. We developed a pneumatic device that simulates an explosive blast by producing pressure transients similar to those observed in a free field explosion, using cell cultures of human brain cells in combination with live-cell Ca^{2+} imaging. Here we show that calcium waves induced by a simulated blast propagate primarily through astrocyte-dependent, purinergic signaling pathways that are blocked by P2 receptor antagonists. In addition, we found increased expressions of a reactive astrocyte marker, glial fibrillary acidic protein (GFAP) and a protease, matrix metallopeptidase 9 (MMP-9). Since human astrocytes differ from rodent and other primate astrocytes structurally and physiologically, their response to simulated blast may also differ. By comparing responses to rat astrocytes, we found that human astrocytes had an increased integrated calcium response and prolonged calcium wave propagation kinetics. This differential sensitivity is an important consideration in studies of TBI treatments and countermeasures, suggesting that greater forces may be needed in rodent model systems to replicate comparable damage to human astrocytes.

Disclosures: **R. Ravin:** None. **P. Blank:** None. **B. Busse:** None. **N. Ravin:** None. **S. Vira:** None. **L. Bezrukov:** None. **H. Waters:** None. **H. Guerrero-Cazares:** None. **A. Quinones-Hinojosa:** None. **P. Lee:** None. **R.D. Fields:** None. **S. Bezrukov:** None. **J. Zimmerberg:** None.

Poster

611. Trauma Mechanisms and Therapeutic Strategies

Location: Halls B-H

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Program#/Poster#: 611.01/DD11

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant NS 40516

VA Merit Grant I01 BX000589

AHA Western States Affiliate Postdoc Fellowship 13POST14810019

Title: Triggering receptor expressed on myeloid cells-2 (TREM2) deficiency worsens outcome after experimental traumatic brain injury

Authors: *M. A. YENARI[1], M. KAWABORI[2], J. KIM[2], Z. ZHEN[2], R. KACIMI[2], C. HSIEH[3]; [1]Neurol, Univ. California, San Francisco and San Francisco Veterans Affairs Med. Ctr., San Francisco, CA; [2]Neurol., [3]Med., Univ. of California, San Francisco & San Francisco VAMC, San Francisco, CA

Abstract: Triggering receptor expressed by myeloid cells-2 (TREM2) is a surface receptor present on microglia and macrophages. It was first described as a receptor of the innate immune system which bound pathogens and led to their phagocytosis. TREM2 deficiency leads to dementia, and we recently showed that its deficiency leads to worsened outcome after experimental stroke. Thus, TREM2 may be an important molecule in the clearance of injured cells paving the way towards recovery and repair. Here, we explored whether TREM2 might also play a role in experimental brain trauma TBI. Male TREM2 knockout (TREM Ko) or wildtype (Wt) mice (n=12/group) were subjected to controlled cortical impact (CCI), then assessed for neurological function at 1, 3, 7 & 14 d later. Mice were assessed for neurological deficit (Bederson score), lateralization on an elevated body swing test, and foot faults from a ladder test. Brains were then harvested for histology. TREM Ko mice had worsened neurological recovery compared to Wt on all three functional studies ($p<0.05$) and had markedly increased lesion volumes (2x larger than Wt, $p<0.01$). This was also associated with reduced resorption of injured brain tissue amongst Ko mice. We also studied Neuro2a cells in combination with BV2 microglia. Microglial treatment with IL-4 induced a M2 (anti-inflammatory) phenotype as determined by arginase 1, CD206 & YM1 induction, and also increased TREM2 expression. When TREM2 was silenced using siRNA, arginase1 induction was decreased while iNOS (M1, pro-inflammatory) was increased. TREM2 silencing in BV2 cells also decreased neuronal phagocytosis in response to microglial activators. These results indicate that TREM2 deficiency worsens outcome from experimental TBI, and that TREM2 plays a role in the phagocytosis of injured brain cells, and leads towards a M2 phenotype.

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Poster

611. Trauma Mechanisms and Therapeutic Strategies

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Program#/Poster#: 611.02/DD12

Topic: C.09. Brain Injury and Trauma

Support: FEDER funds through the Operational Programme Factors Competitiveness - COMPETE

National Funds through FCT - Foundation for Science and Technology

Title: Specific cellular association of liposomes by stem-like cells improves therapeutic efficacy in glioblastoma

Authors: *J. BALÇA-SILVA[1,2], A. DO CARMO[2,3], A. SARMENTO-RIBEIRO[1,2,4], V. MOURA-NETO[5], M. LOPES[2,6], J. MOREIRA[2,6];
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Abstract: Glioblastoma (GBM) is the deadliest astrocytoma. The mean survival time for GBM patients is approximately 14 months under the *gold standard,* temozolomide (TMZ). There are three of the main reasons that make the GBM treatment difficult to surpass: 1) the constitutive activation of important signalling pathways involved in the GBM's proliferative capability, namely the protein kinase C (PKC); 2) the presence of a population of cells with stem-like cell properties (GSCs), which are known to be chemo- and radioresistant; and 3) the existence of the blood-brain-barrier (BBB), that limits the delivery of therapeutic agents.
To overcome these difficulties, we intended to: 1) use a targeted drug delivery liposome that allows BBB passage and reduces the systemic side effects of chemotherapeutics and 2) combine with lower doses of specific drugs, namely tamoxifen (TMX), inhibitor of PKC, and TMZ, that cross the BBB, to potentiate the effect. For that, we used a F3-peptide-targeted sterically stabilized pH-sensitive liposome, which recognizes cell-surface nucleolin (NCL), an overexpressed receptor presented in tumor cells, allowing a drug-specific delivery. These liposomes contained doxorubicin (DXR), an anti-tumor drug with multiple effectiveness, high systemic toxicity and that can not cross the BBB by its own.
Herein, NCL expression and stem-like cell markers were assessed, by flow cytometry, in two GBM cell lines, U87 and GBM11. We also determined the ability of GBM cells to specifically uptake the F3 peptide-targeted liposomes, and further correlated with the expression of stem-like cell markers, by flow cytometry. We assessed the effect of the combined therapy, TMX and

TMZ, with F3 peptide-targeted liposome containing DXR, on PKC expression, by western blot. The cytotoxicity of liposomes combined with TMX and TMZ was further assessed by the MTT assay.
Our results showed that the F3 peptide-targeted liposomes successfully targeted U87 and GBM11 cells, particularly GSCs, but the effect seems to be associated with the NCL overexpression. This approach enabled intracellular delivery of DXR with high specificity, inducing cell cytotoxicity and reducing cell proliferation, when in comparation with non-targeted liposomes. The cell proliferation was further reduced when F3 peptide-targeted liposomes were associated to the TMX and/or TMZ combination.
In this study we showed that liposomes can induce a drug-specific delivery, depending on NCL expression, which emphasized the importance of a personalized therapy. Also, the combination of liposomes with TMX and /or TMZ is a potential strategy to treat GBM.

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Poster

611. Trauma Mechanisms and Therapeutic Strategies

Location: Halls B-H

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Program#/Poster#: 611.03/DD13

Topic: C.09. Brain Injury and Trauma

Support: International Cell Surgical Society Grant

Title: Effective treatment of traumatic brain injury in rowett nude rats with stromal vascular fraction containing adipose derived stem cells

Authors: *S. BERMAN[1], M. BERMAN[1], E. LANDER[2], R. COHEN[3];
[1]Res., Cell Surgical Network, Beverly Hills, CA; [2]Res., Cell Surgical Network, Rancho Mirage, CA; [3]Biol., California State Univ. Northridge, Northridge, CA

Abstract: Traumatic brain injury (TBI) affects about 1.9 million Americans every year with many more cases going unreported. Blast traumatic brain injury (bTBI) has been labeled the signature injury of the wars in Iraq and Afghanistan, and the physiology is similar to that of a TBI sustained in automobile accidents and recreational activities such as American football. TBI can lead to dizziness, loss of motor skills, rapid mood swings, and memory loss. Current treatments for TBI are lacking with most seeking to ameliorate the symptoms of TBI but doing little to actively repair the neuronal damage near the site of injury. This project investigated the hypothesis that stromal vascular fraction (SVF), a rich source of adipose derived and

hematopoietic stem cells, can assist in functional and histological post-TBI recovery. Human SVF was obtained from patient donors via elective liposuction in accordance with Cell Surgical Network protocols and consent of the patients. After extraction, adipose tissue was incubated in a closed sterile container with collagenase for enzymatic digestion of the extracellular matrix. The sample was washed with sterile saline and centrifuged to isolate the SVF. We utilized the acoustic wave technology of the Storz-D-Actor to induce a closed head TBI in adult Rowett Nude (RNU) immunosuppressed rats. A 5.0 bar acoustic wave was delivered to the left frontal cortex in a closed head procedure. Within one hour post-TBI, the freshly harvested SVF containing approximately 500,000 cells suspended in sterile 0.5 ml lactated Ringers solution was incubated with Qtracker 625 cell labeling solution and was administered into the RNU rats via the tail vein. We utilized a second group of RNU rats that received the same bolus of SVF into the tail vein 72 hours post-TBI. Lactated Ringers solution was also administered via tail vein injection into control RNU rats. Rotarod and water maze assays were used to monitor post-TBI symptoms of declining motor skills and spatial memories, respectively. Rats treated immediately after TBI with SVF showed no signs of diminishing motor skills and memory. SVF treatment 72 hours post-TBI helped the rats return to their motor skills baseline only, while control rats treated with Lactated Ringers solution showed a 25% decrease in their motor skills and memory tests 14 days post-TBI. Histological analysis showed the presence of Qtracker labeled cells within the infarct in both SVF treatment groups. However, Qtracker labeled cells were roughly twice as numerous in the group treated with SVF immediately following TBI. Our study suggests that SVF could serve as a potential therapeutic agent in treating patients recovering from TBI.

Disclosures: **S. Berman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Mark Berman MD and Dr. Elliot Lander MD are the founders of the Cell Surgical Network and provided the stromal vascular fraction used for this study but were not present for the animal testing. **M. Berman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Mark Berman MD and Dr. Elliot Lander MD are the founders of the Cell Surgical Network and provided the stromal vascular fraction used for this study but were not present for the animal testing. **E. Lander:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Mark Berman MD and Dr. Elliot Lander MD are the founders of the Cell Surgical Network and provided the stromal vascular fraction used for this study but were not present for the animal testing.. **R. Cohen:** None.

Poster

611. Trauma Mechanisms and Therapeutic Strategies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 611.04/DD14

Topic: C.09. Brain Injury and Trauma

Support: NHMRC

Title: The effects of interleukin-1 receptor antagonist treatment in a mouse model of multitrauma

Authors: *M. SUN[1], R. D. BRADY[2], D. K. WRIGHT[3], S. LIU[1], B. D. SEMPLE[1], T. J. O'BRIEN[1], S. J. MCDONALD[2], S. R. SHULTZ[1];
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Abstract: BACKGROUND: Multitrauma involves injury to at least two body regions, and is prevalent worldwide. Traumatic brain injury (TBI) and bone fracture are two of the most common components of multitrauma. We have recently demonstrated that this injury combination in mice results in worse TBI-related outcomes than an isolated TBI, and that this occurred in the presence of an exacerbated neuroinflammatory response involving significantly elevated levels of interleukin-1β (IL-1β). Accordingly, here we aimed to determine if treatment with an IL-1 receptor antagonist (IL-1ra) would reduce neuroinflammation and improve outcomes in mice given multitrauma. METHODS: 12 weeks old Male C57Bl/6 male mice were randomly allocated to sham (SHAM) or multitrauma (weight-drop TBI and internally-fixated tibial fracture; MULTI) groups. Mice received subcutaneous injection of either 100 mg/kg of the IL-1ra or vehicle (VEH) at 1-, 6-, 24-hours and then daily for 1 week post-injury. A portion of mice were killed at 48 hours, and the remaining mice were given a 12-week recovery period. Cerebral edema and markers for neuroinflammation were assessed in all mice. Behavioral testing, magnetic resonance imaging (MRI), and pentylenetetrazole (PTZ) challenge tests were also conducted in the 12-week recovery mice. RESULTS: IL-1ra-treated MULTI mice displayed less cerebral edema and cortical neutrophils/monocytes infiltration at 48 hours post-injury compared to their VEH-treated counterparts. Preliminary behavior findings suggest that the VEH-treated MULTI mice had social impairments in the 3-chamber task, while the IL-1ra-treated MULTI mice did not. Additional behavior, MRI and PTZ analyses are ongoing. CONCLUSIONS: These preliminary findings indicate that IL-1ra may be an effective treatment in multitrauma featuring TBI and long bone fracture. Studies are ongoing to determine the influence of IL-1ra treatment at additional acute time points and on long-term behavior, neurodegeneration, epilepsy-related outcomes, and fracture healing following multitrauma.

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Poster

611. Trauma Mechanisms and Therapeutic Strategies

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Topic: C.09. Brain Injury and Trauma

Support: Department of Veterans Affairs Merit Award

Craig H. Neilsen Foundation

NY State SCIRB

Title: Transmission from motor cortex to spinal cord neurons and limb muscles in intact and lesioned motor cortex of adult rats

Authors: *H. A. PETROSYAN[1,2], V. ARVANIAN[1,2];
[1]Neurobio. and Behavior, Stony Brook Univ., Stony Brook, NY; [2]Northport VA Med. Ctr., Northport, NY

Abstract: Corticospinal tract is a major descending pathway mediating voluntary movements. The loss of cortical input due to injuries or stroke leads to severe deficits with limited recovery. Objective of the current study was to examine transmission from motor cortex to individual neurons in cervical C4-C5 spinal cord and to forelimb muscles in non-injured and in animals after acute lesion of sensorimotor cortex. We performed systematic quantitative evaluation of synaptic transmission to individual spinal neurons bilaterally and its correlation with responses recorded from forelimb muscles. Number of spinal neurons receiving synaptic inputs from the motor cortex and the ratio of excitatory and inhibitory synaptic inputs were determined in intact and in animals immediately after unilateral lesion of SMC. Results demonstrate that in response to electric stimulation of one hemisphere neuronal responses were recorded bilaterally from cervical spinal cord. Motor evoked potentials (MEPs) form both forelimbs were present in response to the same stimulation, although ipsilateral responses required higher stimulation intensity. On the contralateral to stimulation side the majority of responses were EPSPs with 75.9 ± 0.8% out of all responses, 20.7 ± 0.7% of responses were being IPSPs and small number 3.3 ± 0.4% of responses were mixed responses containing both IPSP and EPSP inputs. On the ipsilateral to stimulation side the distribution of recorded responses was different: number of recorded EPSPs was higher and number of IPSPs was lower compared to contralateral side (90.8

± 0.1% for EPSP and 8.4 ± 0.1 % for IPSP). After unilateral acute lesion of SMC, neither MEPs nor the distribution of intracellularly recorded synaptic responses were significantly changed at the contralateral to stimulation (i.e. ipsilateral to injury) side. However, injury significantly affected both synaptic responses and MEPs recorded from the ipsilateral to stimulation (e.g. contralateral to injury) side. MEPs recorded from contralateral to injury forelimbs were diminished. Importantly neurons on the contralateral to the lesion side of the cord were still responsive to ipsilateral SMC stimulation; however number of these neurons was significantly decreased and the ratio of inhibitory vs excitatory inputs was changed. Inhibitory inputs were decreased compared to before injury. Spared ipsilateral projections to individual spinal neurons, which sustain after unilateral cortical lesions, is a novel observation. These projections are promising candidates for future research and strengthening of these projections may be a target for treatment development after TBI and stroke.

Disclosures: H.A. Petrosyan: None. **V. Arvanian:** None.

Poster

611. Trauma Mechanisms and Therapeutic Strategies

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Dart NeuroScience, LLC

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Title: NMDA receptor-mediated activity is required for neuronal integration after injury in Xenopus visual system

Authors: *T. J. WISHARD[1,2], A. C. GAMBRILL[2], C. R. MCKEOWN[2], H. T. CLINE[2]; [1]Neurobio., UCLA, Los Angeles, CA; [2]Mol. and Cell. Neurosci., The Scripps Res. Inst., La Jolla, CA

Abstract: More than 5 million Americans suffer from traumatic brain injury (TBI), resulting in an approximate social and medical management cost burden of over $800 million per year, however the cellular and molecular mechanisms that mediate recovery from TBI are poorly understood. We previously developed a penetrative brain injury model with a behavioral output

using the visual system of Xenopus laevis. Focal ablation of part of the optic tectum in Xenopus tadpoles results in increased neurogenesis, however the extent to which newly born neurons are integrated into the tectal circuit, and their role in the circuit's recovery are unclear. NMDA receptors (NMDARs) play an important role in synaptogenesis and network maturation in many systems. To test the requirement of NMDARs in new neuron integration and functional recovery in our injury model, we used pharmacological and molecular techniques to block GluN2B-containing NMDARs. Using electrophysiology, we found that blockade of GluN2B with ifenprodil significantly impairs normal synaptogenesis. Using in vivo time-lapse imaging to evaluate the structural plasticity of individual optic tectal neurons, we found treatment with ifenprodil significantly reduced dendritic arbor elaboration. Injury to the optic tectum results in a quantifiable loss of visually-guided behavior in the Xenopus tadpole. Recovery of behavior takes 5-7 days and is dependent on cell proliferation. We tested the requirement of GluN2B-containing NMDARs for behavioral recovery by using morpholinos to acutely knockdown GluN2B-containing NMDARs. We found that loss of GluN2B significantly slowed the time course of behavioral recovery from injury. Our data indicate that GluN2B-mediated activity is required for normal integration of new neurons into the tectal network and is important for the recovery of circuit function after injury.

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Poster

611. Trauma Mechanisms and Therapeutic Strategies

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Topic: C.09. Brain Injury and Trauma

Support: NIH/NINDS NS086570-01 (SHR)

The Shriners Hospitals for Children 85110-PH1-14 (SHR)

Title: Acute administration of endothelial-targeted catalase attenuates neuropathology and cortical microglia activation in traumatic brain injury

Authors: *E. M. LUTTON[1], R. RAZMPOUR[1], M. SEASOCK[1], S. F. MERKEL[1], L. CANNELLA[1], A. M. ANDREWS[1], V. SHUVAEV[2], V. MUZYKANTOV[2], S. H. RAMIREZ[1]; [1]Temple Univ., Philadelphia, PA; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: TBI is a major clinical and social concern that contributes to one third of all injury related deaths in the US. Secondary mechanisms of injury in TBI, such as inflammation, are points at which intervention may improve functional recovery. Current treatment strategies for TBI are supportive, and the pathophysiology is not fully understood; however, evidence suggests that reactive oxygen species (ROS) and oxidative stress propagate blood-brain-barrier (BBB) hyperpermeability and inflammation following TBI. Such inflammation is associated with activated microglia that have been shown to persist for years after injury in the human brain. The novel use of endothelial-targeted catalase in TBI is hypothesized to quench ROS at their source to limit inflammatory activation and protect BBB function. Preliminary data demonstrates a consistent time dependent increase in vascular expression of endothelial ICAM-1 after TBI. To evaluate targeted antioxidant enzyme efficacy in TBI, catalase was conjugated to anti-ICAM-1 antibodies and administered to 6wk old C57BL6 mice 30min after moderate controlled cortical impact injury. Results suggest that administration of catalase targeted to ICAM-1 reduces neuroinflammatory indices and BBB permeability. Specifically, the study of microglia in situ by multiphoton microscopy following TBI revealed that treatment with anti-ICAM-1/catalase attenuates microglia transition to an activated phenotype. These results demonstrate an effective proof-of-concept approach to acute TBI management that may also be applicable to other neuroinflammatory conditions.

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Poster

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Topic: C.09. Brain Injury and Trauma

Support: JSPS KAKENHI Grant Number 15K16374

Title: Behavioral, neuroanatomical, and dna microarray study after neonatal and adult unilateral brain hemisuction mice

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Abstract: Nervous system is generally hard to regeneration, and the brain subjected to damages such as stroke and trauma results in loss of motor and/or sensory functions. It is also recognized that neonatal brain in infants often exhibits remarkably functional recovery even if they subjected to severe damages. However, it is unclear why the neonatal brain recover the neuronal functions. Here, we compared the differences of forelimb motor function, neuroanatomical recovery and gene expressions between infant and adult brain after left cerebral hemisuction. C57/BL6 strain infant (postnatal day7) and young adult (8-10 weeks) mice were subjected to left cerebral hemisuction under sevoflurane inhalation. Then, the mice were examined ladder walking test, which counted the slipping from ladder on forelimb, 4, 8, and 12 weeks later. After that, Fluoro-gold (FG), a retrograde neural tracer, was injected into the grey matter of right C5 - C6 vertebrae for histological evaluation. Another animals were removed the contralateral hemisphere 1 and 4 weeks after injury to survey their transcriptome with a 8 × 60 K mouse whole genome Agilent DNA chip.
In the behavioral test, the neonatal brain hemisuction mice (NBH) gradually improved the walking in a time-dependent fashion although adult brain hemisuction one (ABH) did not. The percentages of the foot slip in NBH were significantly lesser than those in ABH at all time points. FG signals in the contralateral hemisphere well recognized in the forebrain area of neocortex and were greater in the NBH. The transcriptome analysis was determined that 193 and 61 genes in NBH, and 1703 and 98 genes in ABH increased >1.5-fold at 1 and 4 weeks, respectively. Conversely, 990 and 2161 genes in NBH, and 2595 and 1022 genes in ABH decreased <0.75-fold, respectively. These results suggest that the brain in NBH was higher plasticity than that in ABH, and the compensatory neuronal projection from contralateral hemisphere might be important for the motor functional recovery. Now, we have been analyzing the genes that relate to the neuronal projection.

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Poster

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Topic: C.09. Brain Injury and Trauma

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Title: Angiotensin receptor type 1 deficiency or inhibition improves outcome after experimental traumatic brain injury

Authors: J. KIM[1], N. KIM[1], *Z. ZHENG[3], M. JOHNSON[2], M. A. YENARI[1];
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Abstract: Angiotensin II receptor type 1 (ATR1) mediates vasoconstriction, and its inhibition has been widely used to treat hypertension; however, recent work has suggested that it may also modulate apoptosis, and neuroinflammation. Thus, treatment with already available ATR1 blockers may have additional neuroprotective value. We explored the contribution of ATR1 to neuroprotection and brain hemorrhage in a model of TBI. Male, wildtype (Wt) and ATR1 knockout (Ko) mice were subjected to TBI using controlled cortical impact (CCI). Sensorimotor function (adhesive removal & elevated body swing tests), brain hemorrhage and lesion size were assessed out to 14d post insult. Wt mice were also treated with an ATR1 inhibitor (candesartan, 0.1mg/kg IP). ATR1 deficient mice were protected from CCI as evidenced by decreased lesion volumes (decreases of ~40% in lesion size amongst Ko mice, n=6/group, $p<0.05$), improved neurobehavioral outcomes (n=6/group, $p<0.05$) and fewer activated microglia in Ko mice ($p<0.05$). This was also associated with decreased TNF-alpha and IL-6 cytokine expression relative to Wt. Further, the ATR1Ko mice suffered less brain hemorrhage ($p<0.05$) and this was correlated to reduced MMP-9 expression ($p<0.01$). Candesartan similarly protected against brain injury, brain hemorrhage and improved neurological outcome out to 14 days post CCI (n=6/group, $p<0.05$). Candesartan, while often used to treat hypertension in humans, did not reduce blood pressure in mice at the dose studied. These data are consistent with the notion that ATR1 contributes negatively to traumatic brain injury, and its inhibition or deficiency leads to improved outcomes and decreased immune responses. Considering the clinical availability of ATR1 inhibitors, this approach may be a promising novel therapeutic target against TBI and related conditions.

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Poster

611. Trauma Mechanisms and Therapeutic Strategies

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Title: Effects of mild traumatic brain injury on monoamine receptors and anxiety-like behavior in male and female rats: response to post-injury progesterone therapy

Authors: *L. C. FOX[1], G. M. PALMER[1], J. L. SCHOLL[2], M. J. WATT[2], G. L. FORSTER[2]; [2]Basic Biomed. Sci., [1]Univ. of South Dakota, Vermillion, SD

Abstract: Mild traumatic brain injuries (mild TBIs) comprise over seventy-five percent of head traumas in the United States each year, and usually occur in settings of stress (sports, warfare, domestic violence, etc.). Symptoms can persist for weeks, months or even years in some victims, and can include memory difficulties, mood changes, and often generalized anxiety. Both males and females are at risk for mild TBI, but differential outcomes have been observed between males, naturally cycling females, and females taking hormonal contraceptives. Women using pharmacological doses of estradiol and progesterone were found in a 2013 study to experience fewer negative symptoms after injury than women who did not, as well as fewer than males. Our study utilized a rat model to explore the mechanisms behind this sex difference, and any potential benefits ovarian hormones may offer for concussion victims. Male and naturally-cycling female adult rats received a mild TBI via a weight drop mechanism, immediately following an episode of social defeat. This model of paired stress with injury effectively mimics the neurological environment in which most mild TBIs occur. Following assessment of anxiety-like behavior (elevated plus maze, EPM), perfused brains were taken and labeled using immunocytochemistry for serotonin, dopamine and GABA receptors. These neurotransmitters are known to be responsive to changes both in estradiol and progesterone levels. It was found that male rats reliably experience anxiety symptoms following mild TBI, but only females in the diestrus phase of their estrus cycle on the day of testing replicate this. Females in metestrus, estrus and proestrus appear protected, and spend similar times in the EPM open arms to those of uninjured controls. Mechanisms underlying this behavior may involve interactions between ovarian sex hormones and monoamine receptors in the brain. Recognizing this connection and the potential for therapeutic benefit, progesterone (4mg/kg) was administered 3 hours following injury, and then once per day for 5 days afterward. Progesterone treatment did not improve anxiety-like behavior of male rats exposed to mild TBI, suggesting that while progesterone might be protective in females, it may not restore psychosocial symptoms following mild TBI in males. Future work should test different combinations and timings of ovarian sex steroids to determine whether these can prevent psychosocial symptoms from developing following mild TBI in males and females.

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Poster

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Tel-Aviv University, Tel-Aviv, Israel

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Title: Evaluation of a sustained release form of the glucagon-like peptide-1 receptor agonist exendin-4 (PT302) in a mouse model of mild traumatic brain injury

Authors: *D. TWEEDIE[1], Y. LI[1], I. TAMARGO[1], N. GREIG[1], M. BADER[2], L. RACHMANY[2], C. PICK[2], D. KIM[1];
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Abstract: Traumatic brain injury (TBI) is a common event occurring in civilian and military environments as a result of falls, full contact sports, automobile accidents, acts of violence and shockwaves generated by the detonation of high explosives. Primary brain injury includes brain tissue deformation and/or damage. Secondary brain injury involves neuronal excitotoxicity, oxidative stress and inflammatory processes. Currently there is no FDA approved drug for the management of secondary injury events following TBI. Our prior work described cellular and behavioral benefits and reversals of numerous TBI-induced hippocampal gene expressions by the glucagon-like peptide-1 (GLP-1) receptor agonist exendin-4 in both concussive and blast TBI. Here we describe the actions of a sustained release form of exendin-4 (PT302) on TBI-induced behavioral deficits in a closed head weight drop model of TBI. Concussive mild TBI (mTBI) was induced by dropping a 30 g weight from a height of 80 cm onto the heads of anesthetized male mice. Sub-sets of mice were administered a single subcutaneous administration of PT302 (0.024, 0.12 and 0.6 mg/kg) 1 hr after TBI. Pharmacokinetic studies illustrated sustained plasma Exendin-4 levels, biphasically peaking at 2 hr and then 14 days following PT302 dosing, which matched levels observed in human subjects. Assessment of cognitive and anxiety-like behavior was undertaken 7 days post-injury using the Novel Object Recognition, Y-maze and Elevated Plus Maze paradigms. In mTBI+vehicle animals, cognitive deficits were observed while PT302 treatment 1 hr post-injury ameliorated these TBI-induced impairments. Further studies are evaluating dose-dependence and optimization to support the clinical evaluation of PT302 in human TBI.

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Poster

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Topic: C.09. Brain Injury and Trauma

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American Academy of Neurology

Title: Inhibition of eph/ephrin signaling promotes recovery following traumatic brain injury

Authors: S. TENG[1], A. PALMIERI[1], J. PARK[1], R. ZHOU[2], J. ALDER[1], *S. THAKKER-VARIA[1];
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Abstract: Revealing the cellular and molecular pathways underlying neuronal cell death and degeneration after traumatic brain injury (TBI) is critical to identifying novel therapeutics. Eph/Ephrin family members are repulsive to process outgrowth and inhibiting Eph function or expression enhances recovery after spinal cord injury. However, whether blocking Eph receptors signaling promotes regeneration after TBI has not been explored. Our data demonstrate that EphA6 receptor is expressed in the adult brain and is upregulated in the hippocampus following the lateral fluid percussion (LFP) model of TBI. In uninjured EphA6 knockout (KO) mice, neurons grow abnormally exuberant processes, suggesting that EphA6 normally restricts growth of axons and dendrites. In the current study, we have used EphA6 KO mice to examine cellular and behavioral recovery following LFP injury in adult male mice. We have demonstrated that EphA6 KO mice have significantly less neuronal cell death and glial scarring following LFP compared to wildtype (WT) animals as indicated by activated caspase-3 and GFAP immunohistochemistry, respectively. Using EphA6 KO mice crossed to transgenic Thy1-YFP mice to visualize axons, we have determined that the absence of EphA6 signaling enhances regeneration in the cortex. For a more clinically relevant treatment paradigm, we have used soluble antagonist EphA6-Fc or control IgG administered to cortex of WT mice via mini-osmotic pumps for 7 d following LFP. EphA6-Fc treated mice exhibited less cell death and degeneration as demonstrated by FluorojadeC staining relative to IgG treated mice. The mice given EphA6-Fc also had a longer latency to fall on the rotarod 21 d after injury indicating improved motor

function compared to control mice. One ligand for the EphA6 receptor is Ephrin-A5 and there is high expression of Ephrin-A5 in the hippocampus and cortex of adult mice. We have shown that clustered Ephrin-A5 is also inhibitory to process outgrowth in vitro. Furthermore our studies indicate that a protein phosphotyrosine phosphatase inhibitor can rescue growth cone collapse induced by clustered Ephrin-A5. This compound is currently being tested in vivo to determine if it can mimic the effects of the EphA6 KO and EphA6-Fc on cell death and motor function. We are also using clustered EphrinA5-Fc, which acts as an agonist to the EphA6 receptor to see if there are exacerbated cellular and behavioral outcomes after LFP. Together these studies will yield insights into the role of Eph/Ephrin pathways in injury and demonstrate the therapeutic potential of intervention of EphA6 signaling via Fc fusion proteins and pharmacological agents for the treatment of TBI.

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Poster

611. Trauma Mechanisms and Therapeutic Strategies

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Topic: C.09. Brain Injury and Trauma

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Title: Distal axotomy induced retrograde signaling enhances presynaptic excitability onto injured pyramidal neurons

Authors: *T. NAGENDRAN[1,2], R. L. BIGLER[3], R. LARSEN[2,4], B. D. PHILPOT[2,4,5], A. M. TAYLOR[1,2,5];
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Abstract: Distal injury of long pyramidal tracts remodels cortical circuitry and leads to enhanced neuronal excitability, thus influencing recovery following injury. The neuron-specific contributions to this retrograde injury-induced hyper-excitability remain unclear due to the complex cellular composition and connectivity of the CNS. We developed a novel microfluidics-based *in vitro* model system to examine intrinsic synaptic remodeling following distal axotomy

of long projection pyramidal neurons. We found that distal axotomy of rat pyramidal neurons caused dendritic spine loss at synapses onto the injured neurons followed by a delayed and persistent retrograde trans-synaptic enhancement in presynaptic excitability. Further, this hyper-excitability involved the specific elimination of inhibitory presynaptic terminals formed onto dendritic spines. We found that these changes required differential gene expression and axotomy decreased mRNA expression of the secreted factor netrin-1 coinciding with spine loss. Exogenous netrin-1 applied two days after injury normalized this presynaptic hyper-excitability and restored the fraction of inhibitory inputs onto injured neurons. These findings provide new insights of neuron-specific mechanisms that contribute to synaptic remodeling and demonstrate a novel model system for studying the response of pyramidal circuitry to axotomy.

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Poster

611. Trauma Mechanisms and Therapeutic Strategies

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Topic: C.09. Brain Injury and Trauma

Support: DoD in the Center for Neuroscience and Regenerative Medicine (CNRM)

Title: Adult neural stem cell intracerebroventricular transplantation in experimental traumatic brain injury: analysis of the sonic hedgehog mediated regenerative response and neuroinflammation.

Authors: *G. M. SULLIVAN, R. C. ARMSTRONG;
USUHS, Bethesda, MD

Abstract: Neural stem cells (NSCs) hold promise to promote brain repair by replacing lost cells and/or interacting with host cells to modulate the immune response and stimulate endogenous regenerative capacity. Transplantation strategies are challenging for traumatic brain injury (TBI) due to diffuse rather than focal lesions. Therefore, an intracerebroventricular delivery route may be advantageous in TBI patients, and designed via external ventricular drains implanted to control intracranial pressure. To test this concept, NSCs were isolated from adult mouse subventricular zone (SVZ) and transplanted into the lateral ventricle of adult mice at two weeks post-TBI (subacute stage) followed by analysis at four weeks post-TBI. TBI produced reactive

astrogliosis and microglial activation in the corpus callosum (CC) that was significantly reduced by NSC transplantation. We then examined *in vivo* activation of the Sonic hedgehog (Shh) pathway. Shh has important regenerative roles and *Gli1* transcription is an effective readout for canonical Shh signaling. Mouse reporter lines were generated for induced genetic fluorescent labeling of cells actively transcribing *Shh* or *Gli1 in vivo*. Transplanted NSCs from *ShhCreERT2:RosamTmG* mice showed rare *Shh* expression *in vivo*. In host *ShhCreERT2:RosaTdTomato* mice, *Shh* was primarily expressed in neurons, including CC axons. TBI did not induce *Shh* expression in reactive astrocytes or microglia. *Gli1CreERT2:RosaTdTomato* host mice demonstrated only rare Shh activation in CC cells, even after TBI or NSC transplantation. Additionally, NSC transplantation did not activate Shh signaling in host SVZ cells. Therefore, intracerebroventricular NSC transplantation in subacute stage TBI did not stimulate Shh-mediated regeneration yet did significantly suppress neuroinflammation.

Disclosures: G.M. Sullivan: None. **R.C. Armstrong:** None.

Poster

611. Trauma Mechanisms and Therapeutic Strategies

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Title: MicroRNA profiling reveals chronically dysregulated NOTCH1 interactome in the dentate gyrus after experimental TBI

Authors: *N. PUHAKKA[1], A. BOT[2], N. VUOKILA[1], K. DEBSKI[2], K. LUKASIUK[2], A. PITKÄNEN[1];
[1]Univ. of Eastern Finland, Kuopio, Finland; [2]The Nencki Inst. of Exptl. Biology, Polish Acad. of Sci., Warsaw, Poland

Abstract: Each year about 1.5 million people in the United States and 2.5 million in Europe suffer traumatic brain injury (TBI). Life-compromising functional impairments develop in about 43% of injured patients. These impairments can result in several dentate gyrus-regulated disabilities, including memory impairment, depression, and epilepsy. The progression of post-TBI secondary pathology and the evolution of impairments can continue for months. Almost nothing, however, is known about the chronic molecular changes after TBI, and their potential as treatment targets. The present study tested the hypothesis that chronic transcriptional alterations after TBI are under micro RNA (miRNA) control. Expression of miRNAs and their target mRNAs in the dentate gyrus was analyzed using microarrays at 3 months after TBI induced by lateral fluid-percussion injury in adult male Sprague-Dawley rats. Altogether 654 genes were upregulated and 212 downregulated ($p<0.05$). Of 305 miRNAs present on the miRNA-array, 12 were downregulated ($p<0.05$). In parallel, 75 of their target genes were upregulated ($p<0.05$). A bioinformatics analysis of miRNA targets highlighted the dysregulation of the transcription factor NOTCH1 and 39 of its target genes (NOTCH1 interactome). Spearman Rank analysis showed a correlation between miRNA downregulation and the upregulation of *Notch1*. Validation assays confirmed dysregulation of the NOTCH1 interactome, including downregulation of miR-139-5p, upregulation of *Notch1* and its activated protein form (NICD), and positive enrichment of NOTCH1 target gene expression in the microarray dataset. These findings demonstrate that miRNA-based interventions targeting the NOTCH1 interactome have therapeutic potential against dentate gyrus pathology-related morbidities, even in the chronic post-TBI period.

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Poster

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Title: Lithium increases synaptic vesicular proteins, improves neurotransmission and promotes recovery of cognitive function after traumatic brain injury

Authors: *S. W. CARLSON, Y. LI, A. FIRDOUS, X. MA, J. HENCHIR, H. YAN, C. DIXON; Neurosurg. and VA Pittsburgh Healthcare Syst., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Experimental models of traumatic brain injury (TBI) reproduce cognitive impairments and secondary injury sequela reported in TBI patients. Our previous work suggests that reductions in the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, the machinery facilitating vesicular fusion, contribute to impaired neurotransmitter release. TBI significantly reduces cysteine-string protein α (CSPα), an important chaperone protein that facilitates SNARE complex formation. Lithium treatment in naïve rats reportedly increases expression of CSPα. Lithium-treated TBI-injured rats exhibit improved outcome, but the mechanisms mediating the improvement have not been elucidated. The objective of this study was to evaluate the effect of lithium on SNARE protein abundance, evoked striatal neurotransmission, cortical contusion volume, and neurobehavioral function after controlled cortical impact (CCI). Sprague-Dawley rats received CCI (2.7mm) or sham injury, and injected daily (i.p.) with vehicle or 1.0mmol/kg/ml lithium chloride for up to 14d, beginning 5 minutes post-injury. The brains were dissected at 1, 3, 7, 14d post-injury and processed as whole cell or synaptosomal lysates for immunoblotting (n=6/group). Evoked-dopamine release was evaluated using microdialysis at 7d post-injury (n=6-7/group). Lesion volume was assessed at 14d post-injury (n=6/group). Cognitive function was assessed at 10-14d post-injury (n=14-16/group). Lithium significantly attenuated CCI-induced reductions in CSPα and SNARE complexes at multiple time points. Assessment of contusion volume revealed that lithium did not reduce cortical cell loss. At 7d post-injury, lithium significantly improved striatal high-potassium evoked dopamine release. Lithium significantly improved Morris water Maze acquisition and probe trial performance. Taken together, lithium improves SNARE protein abundance, neurotransmission, and cognitive performance following TBI. These findings highlight that lithium increased the abundance of important synaptic proteins that facilitate neurotransmission and promoted functional recovery after TBI.

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Poster

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Topic: C.09. Brain Injury and Trauma

Support: The Japan Foundation for Pediatric Research

Title: Mechanisms of brain edema after injury: Photolysis of a single neuron alters the cytoplasmic Cl^- and cell volume in neighboring neurons.

Authors: *K. EGAWA[1], K. STALERY[2];
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Abstract: Rationale: The mechanisms of cytotoxic edema remain unclear. We investigated the relationship between acute neuronal necrosis and secondary neuronal injury and swelling in neighboring neurons. We studied how acute necrosis of a single neuron affected the chloride concentration in the cytoplasm of surrounding neurons. **Methods:** We used two photon microscopy and the transgenically-expressed chloride fluorophore Clomeleon to explore consequences of death of a neighboring neuron on the intraneuronal chloride ($[Cl^-]_i$) in hippocampal pyramidal cells in organotypic slice cultures. Two photon laser irradiation to the soma of single CA1 pyramidal neuron exclusively lysed the targeted cell. Cell damage was assessed by morphological changes as well as caspase activation using FLICA staining. **Results:** Lysis of the target neuron was followed by an immediate $[Cl^-]$ increase in surrounding neurons; sub-lytic radiation had no effect on neighbors. The time course of $[Cl^-]i$ elevation differed from neuron to neuron, with some neurons exhibiting large increases that persisted over 60 minutes concomitant with caspase activation. The $[Cl^-]i$ increase and subsequent cell damage was exclusively triggered by ionic influx because they were never observed under the perfusion of NaCl free solution. When extracellular NaCl was replaced by Nagluconate, lysis of the target neuron did not cause $[Cl^-]i$ increase or swelling of neighbors, although caspase was activated in cells near the target. In contrast, low Na^+, high Cl^- solutions or NaCl + kynurenic acid had only modest effects on post-lytic $[Cl^-]i$ increase in neighbors. These results highlight the critical role of Cl^- influx for cytotoxic cell swelling and damage after nearby neuronal lysis. The influx pathway of Cl^- is still being elucidated. Pharmacological blockade of $GABA_A$ receptor, cation-chloride co- transporters, and Cl^- channels including sulfate transporters did not reduce the $[Cl^-]i$ increase. The Cl^- channel blockers NPPB and DIDS actually increased neighboring neurons' $[Cl^-]i$ increase and cell volume. **Conclusion:** This novel, readily-studied model demonstrates that cytotoxic edema and injury can occur as purely secondary phenomena in initially uncompromised neurons after acute brain injury. Pharmacological results indicate that swelling

occurs via Cl^- influx pathways that are distinct from known Cl^- channels or transporters. Ion substitution experiments indicate that $[Cl^-]i$ increase is independent of Na^+ influx, suggesting that the counter ion may be potassium. We will continue to utilize this model to explore mechanisms of cytotoxic edema in hopes of developing new therapeutic approaches.

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Poster

611. Trauma Mechanisms and Therapeutic Strategies

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Program#/Poster#: 611.18/EE11

Topic: C.09. Brain Injury and Trauma

Support: VA Merit Award

Title: Regulatory role of nadph oxidase 2 in the polarization dynamics and neurotoxicity of microglia after traumatic brain injury

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Abstract: Several lines of evidence has shown that the prolonged activation of the classic pro-inflammatory (M1) phenotype of microglia may facilitate brain injury progression, and is critically involved in the secondary injury following traumatic brain injury (TBI). Therefore, therapies targeted to skew the microglia polarization toward an alternative, anti-inflammatory (M2) phenotype could provide a potential new therapeutic opportunity for treatment of the injured brain. In the present study, we demonstrated that NADPH oxidase 2 (NOX2) is elevated at 4- and 7-day post TBI in the murine model of controlled cortical impact. To elucidate the role of NOX2 in the regulation of microglia polarization dynamics after TBI, RT-PCR, immunofluorescence staining and flow cytometry for M1 and M2 markers were performed using brain samples collected from wild-type (WT), NOX2-knock out (NOX2-KO), and NOX inhibitor (apocynin)-treated mice following TBI. Our results revealed that at 4- and 7-day post TBI, the NOX2-KO and apocynin-treated mouse brain showed an increase of the anti-inflammatory M2-like microglia and a corresponding decrease of the pro-inflammatory M1-like microglia in the cortex, as compared to the WT vehicle treated animals. In addition, the NOX2-KO and apocynin-treated mice displayed marked down-regulation of the classical NF-κB pathway in microglia, and reduced production of pro-inflammatory cytokines, TNF-α and IL-1β at 4 and 7-day after TBI. Using an *in-vitro* microglia and primary neuron co-cultures system, we

found that microglia from the brain of adult mice at 7-days after TBI show much higher neurotoxicity than the microglia from control and sham mice. Furthermore, the neurotoxicity of microglia from NOX2-KO and apocynin-treated mice is significantly decreased compared with that from WT vehicle-treated mice. Altogether, our results indicate a key role of NADPH oxidase and the NF-κB pathway in regulating the polarization dynamics of microglia and their neuronal toxicity after TBI, and suggest that targeting NADPH oxidase and the NF-κB pathway may be an effective way to ameliorate inflammation and improve outcome following TBI.

Disclosures: **J. Wang:** None. **M. Ma:** None. **K. Dhandapani:** None. **D. Brann:** None.

Poster

611. Trauma Mechanisms and Therapeutic Strategies

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Topic: C.09. Brain Injury and Trauma

Support: German Research Foundation (DFG: CRC 1080, TP A7)

Focus Program Translational Neuroscience (FTN), JGU University Mainz

Title: Impaired synaptic long-term potentiation and changes in protein degradation systems in a mouse model of focal traumatic brain injury in somatosensory cortex

Authors: *T. MITTMANN[1], L. K. FELDMANN[2], F. LE PRIEULT[2], V. FELZEN[3], S. THAL[4], K. ENGELHARD[4], C. BEHL[3];
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Abstract: Traumatic brain injury (TBI) is a leading cause of death in Europe and the US and a challenge to develop new strategies for therapeutical treatments. Processes of functional reorganization and adaptation of neurons following TBI are still not fully understood. The present study evaluated the effects of an unilateral TBI on long-term synaptic plasticity at the border of the lesion after a survival time of 1-2 days in the ipsilateral and contralateral hemisphere. In parallel, we studied protein homeostasis post-TBI by evaluating changes in protein degradation systems. TBI was induced by a controlled cortical impact (CCI) in somatosensory cortex of anaesthetized mice (P18-P21). Acute brain slices of the somatosensory cortex were prepared after a survival time of 1-2 days. A perforated 32 channel-multielectrode array (pMEA) system was used to stimulate and record extracellular field potentials (FPs) in acute brain slices. Late long-term potentiation (L-LTP) was induced by theta-burst-stimulation

(TBS) and recorded for 3 hours. Immunohistochemistry and Western blots were performed to evaluate alterations in the expression of specific autophagic marker proteins LC3, p62 and BAG3. The autophagic flux was measured by incubating acute brain slices with the lysosomal blocker chloroquine and subsequent detection of accumulated LC3II. We also tested the proteasomal activity as well as the expression of plasticity-related proteins (PRP). The MEA recordings revealed a strongly impaired L-LTP in the ipsilateral cortex at 1-2 days post-lesion compared to sham-operated controls. L-LTP was not affected in the homotopic area of the contralateral hemisphere. Interestingly, the expression of PRPs (CaMKIIα) was significantly reduced in the ipsilateral hemisphere of TBI animals. In parallel we observed an increased autophagic flux and a reduced proteasome activity in the ipsilateral hemisphere. Interestingly, FPs recorded with the pMEA in untreated control mice and in presence of autophagy activators (rapamycin) and/or blockers of protein degradation pathways (MG132) mimicked the missing L-LTP of TBI treated animals. These data indicate that TBI in somatosensory cortex impairs L-LTP after 1-2 days, and it is associated with a dysregulation in the expression of CaMKIIα and an altered activity of protein degradation systems. Our pharmacological experiments indicate that the homeostatic regulation of the somatosensory cortex after TBI by the bilateral change in the activity of autophagy and proteasome pathways could mediate the altered L-LTP following TBI. This could be an important adaptive mechanism for functional recovery of the brain after a focal cortical injury.

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Poster

611. Trauma Mechanisms and Therapeutic Strategies

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Topic: C.09. Brain Injury and Trauma

Support: NCTR Protocol E-7584.01

Title: High speed and biaxial stretch as *In vitro* models of traumatic brain injury to the blood brain barrier

Authors: *H. ROSAS-HERNANDEZ[1], E. CUEVAS[1], S. M. LANTZ[1], S. Z. IMAM[1], N. STURDIVANT[2], K. BALACHANDRAN[2], W. SLIKKER, Jr[1], M. G. PAULE[1], S. F. ALI[1]; [1]Natl. Ctr. For Toxicological Res., Jefferson, AR; [2]Dept. of Biomed. Engin., Univ. of Arkansas, Fayetteville, AR

Abstract: Traumatic brain injury (TBI) is one of the major causes of disability in the United States. It occurs when external mechanical forces induce brain damage as a result of impact, penetration, and/or rapid acceleration/deceleration that cause deformation of brain tissue. TBI is also associated with alterations of the blood-brain barrier (BBB), a structure that protects brain tissue from substances circulating in the blood. The BBB consists mainly of brain endothelial cells (BECs). Due to the high incidence and drastic consequences of TBI, it is important to understand the critical events that accompany damage in order to develop effective treatment approaches. Using primary rat BECs as an *in vitro* BBB model, the effects of two different types of stretch that mimic aspects of TBI at 0, 5, 10 and 15% perturbation were characterized. Deformation due to biaxial stretch (BS) was achieved by infusing pressurized gas into flexible bottom culture plates using a commercially available system. Deformation due to uniaxial high-speed stretch (HSS) was achieved by moving a linear actuator, coupled to a polydimethylsiloxane chip on top of a silicone membrane at a strain rate of 100 s^{-1}. Live/dead cells, LDH release, caspase 3/7 staining and nitric oxide (NO) production were evaluated 24 hours after a single stretch episode. BS affected the BECs under all conditions tested, inducing a deformation-dependent decrease in NO production, increase in LDH release, cell death and activation of caspase 3/7, suggesting the induction of apoptosis. On the other hand, HSS increased LDH release only at 15% stretch and increased cell death and caspase 3/7 at 10 and 15% stretch. In summary, some of the events that occur in the BBB after TBI were successfully replicated *in vitro* using BS and HSS and the severity of the TBI produced *in vitro* depends on the degree and orientation of cellular deformation. These data support the use of BS and HSS as valuable tools in the study of TBI in vitro by defining stretch intensities. This method may also be useful in evaluating potential drug treatments for this condition.

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Poster

611. Trauma Mechanisms and Therapeutic Strategies

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Topic: C.08.Stroke

Support: KAKENHI (15K16361)

Grant-in-Aid for Scientific Research on Innovative Areas (Adaptive Circuit Shift)

Title: Selective blockade of the corticorubral tract during intensive forelimb rehabilitation in rats with capsular hemorrhage

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Abstract: Reorganization of the residual neuronal circuits is the key for the poststroke recovery, and it can be promoted by intensive rehabilitation. We reported that forced impaired limb use (FLU) increased the sprouting of the cortico-rubral tract after intracerebral hemorrhage (ICH) that was responsible for the recovered forelimb functions by FLU (Ishida et al., J. Neurosci 2016). However, it is still unclear whether the cortico-rubral tract is involved in the relearning of forelimb functions. In the present study, we investigated the role of the cortico-rubral tract during the period of rehabilitation after ICH in rats.
To block the cortico-rubral pathway during rehabilitation, adult male Wistar rats were injected with two viral vectors: lentivirus vector (NeuRet-TRE-EGFP.eTeNT) was injected into the unilateral red nucleus and adeno-associated virus vector (AAVdj-CaMKII-rtTAV16) was into the ipsilateral motor cortex. Thus, synaptic transmission of the double-infected cortical neurons could be selectively blocked by doxycycline (DOX) administration. Four weeks after the virus injections, ICH was made by collagenase injection into the internal capsule ipsilateral to virus injections.
Half of ICH rats were forced to use their impaired forelimb by one-sleeve cast (FLU) for 7 days from 24 hours after ICH. To block synaptic transmission in the cortico-rubral tract, DOX was orally administered for all rats during FLU. Skilled reaching task was conducted as behavioral assessment for forelimb use at 10-12 and 26-28 days after ICH.
All rats with ICH demonstrated severe impairment of motor function at 24 hours after the lesion. At 10-12 days after the lesion, ICH group without FLU still showed apparent impairment of reaching function. FLU-treated ICH group exhibited substantial recovery of forelimb function. This FLU effect on functional recovery after ICH was not affected by DOX administration during rehabilitation (FLU).
Data indicate that selective blockade of the cortico-rubral tract during FLU did not affect the regain of the forelimb function in an acute phase after ICH, suggesting the compensative recruitment of other descending tract such as cortico-reticulo-spinal pathway rather than the cortico-rubral tract.

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Poster

611. Trauma Mechanisms and Therapeutic Strategies

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Title: An early driver of traumatic brain injury that can be effectively blocked by antibody

Authors: *A. KONDO[1], K. SHAHPASAND[2], O. ALBAYRAM[2], R. MANNIX[3], W. MEEHAN[3], X. ZHOU[2], K. LU[2];
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Abstract: Traumatic brain injury (TBI) is common in contact sport athletes and blast-exposed veterans. Repetitive mild TBI (rmTBI) or even single moderate/severe TBI (ssTBI) is associated with neuropsychiatric symptoms and cognitive disability, which can cause chronic traumatic encephalopathy (CTE), or increase Alzheimer's (AD) risk, but no treatment is available. Extensive tangles made of phosphorylated tau (p-tau) are identified in CTE brains, similar to tauopathy in AD. Since known tau pathologies are not obvious acutely after closed head TBI, whether tauopathy is an end result or early driver of TBI pathology is unclear. Establishing the early role of tau after TBI could result in new treatments, especially since tauopathy spreads and is reduced by tau immunotherapy. *cis*, but not *trans*, p-tau is an early pathogenic conformation in AD, but its role in TBI is unknown. Here we identify cis p-tau as an early central mediator of brain injury after closed head TBI that is effectively stopped by antibody (Kondo et al., 2015, Nature 523: 431-6). We find robust *cis*, but not *trans*, p-tau in sport- and military-related human CTE brains and their TBI mouse models. Upon stress *in vitro*, neurons robustly produce *cis* p-

tau, which disrupts the microtubule network, interrupts mitochondrial transport in neurites, spreads to other neurons, and leads to massive apoptosis. This process, termed "cistauosis", is effectively blocked by *cis* p-tau antibody (mAb), but amplified by *trans* mAb. In mouse models, ssTBI or rmTBI causes strong and persistent induction of *cis* p-tau induction within 12-24 h post-injury, leading to cistauosis in brains. Treating mice with *cis* mAb after ssTBI effectively prevents induction of *cis* p-tau and cistauosis, and also fully reverses TBI-related anxiety/risk-taking behavior. Thus *cis* p-tau appears to be an early driver of brain injury and tauopathy after TBI, but is effectively blocked by mAb. These insights indicate a novel disease mechanism and a potential new therapy for TBI.

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Poster

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Title: Injury leads to the appearance of cells with molecular characteristics of both astrocytes and microglia in mouse and human brain

Authors: M. PEKNA[1], U. WILHELMSSON[1], D. ANDERSSON[1], Y. DE PABLO[1], R. PEKNY[1], A. STAHLBERG[1], J. MULDER[2], N. MITSIOS[2], T. HORTOBAGYI[3], *M. PEKNY[1]; [1]Univ. of Gothenburg, Gothenburg, Sweden; [2]Karolinska Institutet, Stockholm, Sweden; [3]Univ. of Debrecen, Debrecen, Hungary

Abstract: Microglia perform many functions in healthy and diseased brain, ranging from maintenance of homeostasis and regulation of neural plasticity to trophic support and neuroprotection. Although experimental evidence suggests that microglia are not a homogenous

cell population, the knowledge about their functional diversity and its molecular basis is limited. We used single-cell gene expression profiling of freshly isolated cells from uninjured mouse hippocampus and hippocampus after partial deafferentation to assess the heterogeneity of microglia/monocytes and determine their response to injury. We found that in individual cells, microglial markers *Cx3cr1, Aif1, Itgam* and *Cd68* were co-expressed. In the absence of injury, *Cx3cr1* and the astrocyte marker *Gfap* were expressed in two non-overlapping populations of cells. Injury led to the co-expression of these markers both in the injured and contralesional hippocampus. Exposure to lipopolysaccharide (LPS) increased the fraction of cultured cells co-expressing *Cx3cr1* and *Gfap*. Cells co-expressing astrocyte and microglia markers were also detected in sections from human brain affected by stroke, Alzheimer's disease and Lewy body dementia. Our findings indicate that injury and chronic neurodegeneration lead to the appearance of cells that share molecular characteristics of both microglia and astrocytes, two cell types with different embryologic origin.

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Poster

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Topic: C.09. Brain Injury and Trauma

Support: NCTR Protocol E-7584.01

Title: Traumatic brain injury induces astrocytic cell death *In vitro*

Authors: *S. F. ALI[1], H. ROSAS-HERNANDEZ[1], E. CUEVAS[1], S. M. LANTZ[1], B. L. ROBINSON[1], S. Z. IMAM[1], N. STURDIVANT[2], K. BALACHANDRAN[2], J. KANUNGO[1], A. BIRIS[3], W. SLIKKER, Jr[1], M. G. PAULE[1];
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Abstract: Traumatic brain injury (TBI) remains one of the major causes of death and disability. Due to the heterogeneity of its causes, the events that occur after TBI can be quite varied and are not well understood. Regardless of the cause, deformation of the brain tissue leads to neuronal and glial cell death as well as other cellular and molecular responses. Understanding the

subsequent events followed by TBI is extremely important for development of therapeutic approaches. The use of *in vitro* systems allows for the study of specific aspects of TBI. The aim of this study was to characterize the effects of TBI on rat astrocytes using two different *in vitro* membrane perturbation systems at 0, 5, 10 and 15% deformation. TBI was simulated by biaxial stretch (BS) of astrocytic cellular matrices *in vitro* by infusing pressurized gas onto flexible bottom culture plates using a commercially available system. Deformation due to uniaxial high-speed stretch (HSS) was achieved by moving a linear actuator, coupled to a polydimethylsiloxane chip on top of a silicone membrane at a strain rate of 100 s^{-1}. Live/dead cells, LDH release, caspase 3/7 staining and nitric oxide (NO) production were evaluated 24 hours after a single stretch injury. BS induced an increase in LDH release, cell death and activation of caspase 3/7 at 10 and 15% stretch, without changes in NO production. HSS increased LDH release, cell death and caspase 3/7 only at 15% stretch, suggesting that the damage induced by BS is achieved at lower percentages of stretch than that induced by HSS. In summary, we have demonstrated that BS and HSS can be used as models to study the effects of TBI on astrocyte viability *in vitro*. Further experiments are underway to evaluate the effects of TBI-like cellular perturbation on astrocyte metabolism and activation and the impact that these events may have on different brain cell types.

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Poster

611. Trauma Mechanisms and Therapeutic Strategies

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Title: The protective effect of glutamate scavenger combining adenosine A2A receptor agonist CGS21680 against severe traumatic brain injury in mice

Authors: Y.-L. NING[1], N. YANG[1], Z.-A. ZHAO[1], W. BAI[1], H.-K. TIAN[2], J.-S. DIAO[1], X.-L. REN[1], R.-P. XIONG[1], X. CHEN[1], P. LI[1], Y. ZHAO[1], *Y. ZHOU[1];
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Abstract: The adenosine A2A receptor (A2AR) is commonly believed to play an anti-inflammatory role. However, our previous work on moderate traumatic brain injury (TBI) in mice showed that only in the presence of low concentrations of glutamate can the A2AR agonist CGS21680 exert neuroprotective effects, and high concentration of local glutamate switches the effects of A2AR activation from anti-inflammatory and neuroprotective to deleterious. While in a severe traumatic brain injury model, the glutamate levels in the cerebrospinal fluid (CSF) maintained at a high level within 24h. Therefore, there was not a time window to treat the mice with CGS21680 to protect them from brain damage. In the present study we investigated the effects of glutamate scavenger oxaloacetate (OX) combining CGS21680 treatment against severe TBI. Mice were treated with OX intravenously at 1h after severe TBI. The glutamate levels in CSF decreased from 5.707 times to control group to 2.45 times to it at 5h post TBI, and at this time point mice were injected intraperitoneally with CGS21680. At 24h after TBI, nuerological deficit, brain edema and the proinflammatory cytokine TNF-α and IL-1β production were attenuated significantly in OX combining CGS21680 treated mice. Next, to investigate whether the combined treatment is available when CSF glutamate levels were higher and brain insult has been progressed, we treated mice with OX intravenously at 8h after TBI. At 12h after TBI, when the glutamate levels in CSF decreased from 9.377 times to control group to 3.96 times to it, mice were administrated with CGS21680 by i.p. injection. The above brain damages at 24h post TBI were also attenuated in the combined treatment group. Furthermore, the combined treatment exhibited better therapeutic effect than single oxaloacetate treatment did. Taken together, our results demonstrated that A2AR agonist CGS21680 treatment may exert neuroprotective effect against sever TBI when the CSF glutamate level decreased to the range of 2.45 to 3.96 times of that in control group by the impact of OX. Furthermore, no matter the injuries were in early stage or has been progressed, CGS21680 could perform a neuroprotective role, provided the glutamate were maintained at this level by the application of OX, and the protective effects preceded that of oxaloacetate treatment along. The results not only provided a potential therapeutic strategy for the treatment of severe TBI, but also proved the hypothesis that local glutamate levels in the brain dictates the A2AR effects on brain damage outcome.

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Poster

611. Trauma Mechanisms and Therapeutic Strategies

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Topic: C.09. Brain Injury and Trauma

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Title: Evaluation of the effect of THSG on traumatic brain injury

Authors: *Y.-H. CHEN[1], L.-Y. YANG[2];
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Abstract: Traumatic brain injury (TBI) is one of the leading global health issues and has affected at least 10 million people worldwide every year. TBI has placed a tremendous financial burden on the National Healthcare System, the TBI patients and the caring family members. Although lots of efforts have been devoted to the TBI research, there are still no effective therapeutic treatments for TBI. Previous studies have shown that 2,3,5,4'-tetrahydroxystilbene-2-O-beta-D-glucoside (THSG) exerts a protective effect on rat hippocampal neurons against staurosporine-induced neurotoxicity. It has also been reported that THSG inhibits doxorubicin-induced cardiotoxicity via suppression of the reactive oxygen species (ROS) production and the apoptotic signaling. However, the effect of THSG on TBI remains unclear. In this study, we test the hypothesis that THSG attenuates the degree of TBI. We induced TBI on one side of the brain using the cortical impactor and then administered THSG (30 mg/kg, ip.) or vehicle to mice immediately and 12 hr after TBI. Animals were sacrificed 24 hr following TBI. The injured brain and the intact brain were harvested separately and then protein was extracted and processed for the evaluation of p53 and CRMP-2 using the western blotting technique. Our preliminary results showed that TBI induced the expression of p53 and promoted the cleavage of CRMP2. Application of THSG significantly reduced TBI-induced expression of p53 and the cleavage of CRMP2 ($p < 0.05$). These findings indicate that THSG exerts a beneficial effect on TBI animals and strongly suggest that THSG has a great potential to become a novel and effective treatment for TBI patients.

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Poster

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Support: NIH 1DP2CA195763-01

Title: *In vitro* exosome trafficking across the blood-brain barrier

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Abstract: Therapeutic delivery to the central nervous system (CNS) remains a major challenge in the treatment of neurological disorders. This is partly due to the presence of the blood-brain barrier (BBB), a dynamic interface that restricts and controls the passage of substances between the peripheral vascular circulation and the CNS. Different nanocarrier strategies have been explored to improve delivery of therapeutic agents across the BBB. Recent studies have shown that cell-derived vesicles, particularly exosomes, are increasingly recognized as an attractive vehicle for targeting drugs to the brain. However, whether or how they cross the BBB remains to be fully elucidated. Here, we investigated exosome trafficking in brain microvascular endothelial cells (BMECs) *in vitro* under conditions that mimic the healthy and inflamed BBB *in vivo*. Exosomes were engineered to carry *Gaussia* luciferase (Gluc) and Transwell assays were employed to study exosome trafficking *in vitro*. Results suggested that luciferase-carrying exosomes can cross a BMEC monolayer under stroke-like, inflamed conditions (TNF-α activated) but not under healthy conditions ($p < 0.05$). Confocal microscopy demonstrated that exosomes are internalized by BMECs through clathrin-mediated and caveolae-dependent endocytosis, co-localize with early and late endosomes, and are partly exocytosed across the BMECs, in effect primarily utilizing the transcellular route of crossing. Furthermore, approximately one third of the total crossing of the BMECs was due to the paracellular route of passive diffusion through intercellular junctions between BMECs treated with TNF-α. Taken together, these results indicate that cell-derived exosomes can cross the BBB model under stroke-like conditions *in vitro*, and this system can be used to examine whether or not exosomes can be utilized to deliver therapeutic agents to specific regions of the injured brain. Our study provides insights for further development of engineered exosomes as drug delivery vehicles or tracking tools for treating or monitoring brain diseases in the near future. (Supported by NIH 1DP2CA195763-01)

Disclosures: **C. Chen:** None. **C. Wong:** None. **L. Liu:** None. **W. Zhao:** None.

Poster

611. Trauma Mechanisms and Therapeutic Strategies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 611.28/FF3

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant R03NS091699

NIH Grant P30NS055077

Title: C3 transferase gene therapy for neuroprotection and axon regrowth

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Abstract: The bacterial exoenzyme C3 transferase (C3) is a promising therapy for neuroprotection and axon regeneration. However, effects are limited by C3's short duration of action and poor distribution to injured neurons. To address this issue, adeno-associated viral (AAV) vectors tagged with green fluorescent protein (GFP) were engineered to express an endogenous (e) and a secretable/permeable (sp) C3 to allow for long-term and widespread distribution of the C3 protein. C3 gene therapy was delivered via intravitreous injection in the rat optic nerve crush (ONC) injury model. Rats were treated with AAV-eC3GFP or AAV-spC3GFP, or served as controls receiving AAV-eGFP, AAV-spGFP, vehicle alone, or no intervention. At 4 or 8 weeks, RGC survival was quantified following immunostaining of the retina with an RGC-specific Brn3a antibody. The optic nerve was labeled with the anterograde tracer, cholera toxin subunit B, and underwent tissue clearance to allow detailed visualization of the trajectories of regenerating axons through the whole optic nerve. In the control group, only 6% of RGCs survived 8 weeks after injury, whereas treatment with AAV-eC3GFP protected 45% of RGCs from dying, and the widespread distribution by AAV-spC3GFP kept a remarkable 74% of RGCs alive. In addition, robust long-distance axon regeneration was observed at 4 weeks after ONC in both treatment groups compared to controls, with a significant 38- and 24-fold increase in axon regeneration at 1 mm past the crush site for AAV-eC3GFP ($p=0.005$) and AAV-spC3GFP ($p=0.02$), respectively. The neuroprotective studies and three-dimensional analysis of axon regeneration through the transparent optic nerve reveal that this novel C3 gene therapy approach greatly enhances RGC survival and axon regeneration after optic nerve injury. C3 gene therapy could be applied to benefit other neurodegenerative disorders.

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Poster

611. Trauma Mechanisms and Therapeutic Strategies

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Program#/Poster#: 611.29/FF4

Topic: C.09. Brain Injury and Trauma

Support: State of Florida #140: USF Health Veteran PTSD and Traumatic Brain Injury Study

Title: Exercise prior to traumatic brain injury increases cerebral blood flow and improves functional outcomes

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Abstract: Traumatic brain injury (TBI) affects 1.7 million people in the U.S which results in approximately 52,000 deaths each year. Treatments for TBI are limited to managing symptoms and physical therapies which include exercise. The use of exercise before and after TBI has been shown to reduce functional deficits, but the mechanism of action of exercise remains unclear. TBI causes significant damage to the cerebral vasculature system, hence protecting the vascular system may have beneficial effects. We hypothesize that exercise strengthens and preserves the integrity of the cerebrovasculature as a mechanism of action for ameliorating the TBI-induced functional deficits. To test this hypothesis, we examined the role of exercise prior to TBI in enhancing angiogenesis/ vasculogenesis in cerebrovascular system. Adult Sprague-Dawley rats were exposed to one hour forced running wheel exercise daily for 3 days, then were subjected to moderate control cortical impact (CCI) model of TBI. Laser Doppler readings were recorded at baseline and immediately after TBI. Behavior tests, including neurological exams, elevated body swing test (EBST), and Rotarod test were evaluated at day 1 and day 7 after TBI. Learning and memory function was measured through radial arm water maze (RAWM) at day 7 after TBI for 3 days. The results showed a significant increase in cerebral blood flow of 35% and 105% at baseline and after TBI respectively in exercised animals compared to non-exercised animals (p's <0.05). The neurological scores and Rotarod exams, but not EBST were significantly improved in TBI-exercised group compared to TBI non-exercised group (p's <0.05). Moreover, the TBI-exercised animals performed significantly better in learning and memory assessment compared to TBI non-exercised animals (p's <0.05). Preliminary immunohistochemical analyses revealed no significant differences in CD34 and vWF expression, but trends of increased Ang-1 and Ang-2, while decreased VEGFR2 expression were recognized in the peri-impact area. In conclusion, the findings showed that exercised may improve both motor and cognitive deficits induced by

TBI by enhancing cerebral blood flow, and possibly remodeling of the angiogenic and vasculogenic structures.

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Poster

611. Trauma Mechanisms and Therapeutic Strategies

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Program#/Poster#: 611.30/FF5

Topic: C.09. Brain Injury and Trauma

Support: NS095029

Title: Mutant tissue plasminogen activator attenuates white matter injury and improves long term neurological outcomes in a mouse model of traumatic brain injury

Authors: *H. PU[1], X. JIANG[1,2], Y. SHI[1], J. LIU[2], H. MOU[2], S. LUO[2], W. ZHANG[2], W. LI[2], L. ZHANG[1], Y. WANG[2], R. STETLER[1,2], Y. GAO[1,2], X. HU[1,2], J. CHEN[1,2];
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Abstract: Objectives: Traumatic Brain Injury (TBI) is a neurological disorder that leads to long term motor and cognitive dysfunction. Tissue Plasminogen Activator (tPA) has been reported to attenuate motor and cognitive deficits resulting from ischemic injury that shares similar cell death mechanisms with TBI. However, the use of tPA as a neurorestorative agent in clinic may raise concerns, because tPA is also known to cause blood brain barrier damage through its protease actions. In this study, we investigated the long term neuroprotective effects of protease-inactive mutant tPA (tPAm) against TBI.
Methods: TBI was induced in adult male C57BL/6J mice using a controlled cortical impact (CCI) model, and the animals were then randomly assigned to sham, control, 0.9%Nacl and tPAm groups. tPAm was delivered intranasally 1h after CCI and repeated every day for 14 days. For outcome assessments, sensorimotor deficits (rotarod test, cylinder test, foot fault test) were determined at 3-35 days after TBI; cognitive deficits (water maze) was determined at 22-27 days after stroke. The neuronal tracer biotinylated dextran amine (BDA) was injected into the uninjured left motor cortex (M1, 21 day after CCI) to anterogradely label the corticorubral tract and the corticospinal tract. The integrity of myelin sheaths and axons were assessed by expression of myelin basic protein (MBP, Western blots), SMI32 (non-phosphorylated neurofilament), CNPase and NF200. Furthermore, the conductivity of the myelinated nerve

fibers in the corpus callosum surrounding brain regions were evaluated by assessing compound action potentials (CAPs) using electrophysiology.
Results: Our results showed that tPAm attenuated short and long term (up to 35 days after TBI) motor deficits as determined using rotarod test, cylinder and foot fault test. In addition, tPAm significantly improved cognitive performance of TBI mice in the Morris water maze tests at 22-27 days post-TBI. However, tPAm failed to significantly reduce the size of cortical lesion at 35 days after TBI. In contrast to the ineffectiveness in sparing cortical tissue loss, tPAm preserved the integrity of the myelin sheath by preventing the loss of MBP, reducing axonal damage and promoting axonal sprouting. tPAm also promoted functional integrity of white matter, as demonstrated by increased velocity and amplitude of CAP conduction of myelinated nerve fibers in corpus callosum.
Conclusions: In concert, our results suggest that tPAm improves long term neurological outcomes of TBI mice through protection against white matter injury. These observations promote clinical investigations on tPAm as a potential therapeutic agent for TBI patient.

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Poster

612. Molecular Therapeutic Strategies of Spinal Cord Injury

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 612.01/FF6

Topic: C.09. Brain Injury and Trauma

Title: Intrinsic microtubule stabilization by Epothilone B promotes axonal regeneration after spinal root avulsion

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Abstract: A spinal root avulsion injury disconnects the ventral and dorsal spinal roots with the spinal cord. Due to the limited intrinsic axonal extension speed and the inhibitory environment in the CNS/PNS transitional zone (TZ), the time required for axonal regeneration is prolonged, which consequently leads to muscle atrophy and motor dysfunction. Previously, we demonstrated that blocking the inhibitory chondroitin sulfate proteoglycan (CSPG) receptor protein tyrosine phosphatase-σ (PTP-σ) with a small peptide, namely intracellular sigma peptide (ISP) induced more and faster axonal regrowth and enhanced motor functional restoration. After axotomy, the assembly of a new growth cone and continuous axonal extension requires

reconstruction of cytoskeleton and efficient axonal transportation. Microtubule stabilization has been reported to facilitate axonal regeneration in CNS, probably via favoring polymerization of tubulin dimers.
In the present study, we asked whether intrinsic microtubule stabilization by Epothilone B (EpoB) facilities axons to penetrate the CNS/PNS TZ and eventually promotes motor functional recovery after spinal root avulsion. Adult rats underwent cervical ventral root avulsion followed with re-implantation before being treated with EpoB. Motor functional recovery was remarkably enhanced by the administration of EpoB, reflected by the significantly increased Terzis grooming test score (Mann-Whitney U test, $p<0.05$). This was further confirmed by electromyography. At 6 weeks post surgery, higher electrical responses were recorded in the animals treated by EpoB, while spontaneous potentials, generated by denervated muscle fibers, were reduced. At 4 and 6 weeks post surgery, examination of the regenerated axons in the CNS/PNS TZ leaded to the observation that EpoB facilitated direct axonal elongation, instead of meandering courses found in control animals. In addition, EpoB treatment increased the number of ChAT-positive axons in the TZ. These results provide evidences for that microtubule stabilization by EpoB is able to promote axonal regeneration and functional recovery after spinal root avulsion.

Disclosures: H. Li: None. **W. Wu:** None.

Poster

612. Molecular Therapeutic Strategies of Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: Towards Translation Capacity Building 2013, Spinal Cord Injury Network Australia and New Zealand

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Title: Systemic delivery of a Connexin43 hemichannel blocking mimetic peptide improves functional recovery following spinal cord injury in rats

Authors: *Y. MAO[1], R. TONKIN[2], T. NGUYEN[1], S. O'CARROLL[3], L. NICHOLSON[3], C. GREEN[3], G. MOALEM-TAYLOR[2], C. GORRIE[1];
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Abstract: A major challenge in the management of acute spinal cord injury (SCI) is the development of an effective treatment that can be delivered to patients in a timely manner following a traumatic accident. We have shown previously that a mimetic peptide, Peptide5, matching extracellular loop two of the gap junction protein Connexin43 reduces tissue damage and improves functional outcomes when delivered directly to a SCI lesion site. We have now investigated whether systemic (intraperitoneal) delivery results in similar outcomes. Intraperitoneal injections of Peptide5 or control scrambled peptide were given immediately after a mild contusion injury in rats, with injections repeated at 2 and 4 hours post-injury. Rats were then assessed for locomotor recovery and pain hypersensitivity and euthanised at 8 hours (n=8), 2 weeks (n=32) or 6 weeks (n=32) post-injury. The behavioural data of open field and error ladder tests showed an improvement in hindlimb locomotor function in Peptide5 treated rats between 3 and 6 weeks following injury ($p<0.05$). In addition, there was a reduction in at-level mechanical allodynia from 1 week post-injury ($p<0.05$). Immunohistochemistry showed that Peptide5 treatment reduced the expression level of Connexin43 and increased the proportion of phosphorylated Connexin43 protein level at 8 hours after injury compared to the control treatment group ($p<0.05$). At 2 and 6 weeks, lesion size, astrocytic and activated macrophage and microglial responses, as well as neuronal cell loss compared to the controls ($p<0.05$). These results suggest that Peptide5 administered systemically modulates the pathological opening of Connexin43 hemichannels at the lesion site to ameliorate the damage resulting from SCI and has a significant effect on improving functional outcomes.

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Poster

612. Molecular Therapeutic Strategies of Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: NRF Grant 2015H1D3A1066543

Title: Matrix metalloproteinase-8 inhibition prevent disruption of Blood-spinal cord barrier and attenuate inflammation in rat model of spinal cord injury

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Abstract: Objective: - After spinal cord injury (SCI), the degradation of tight junction (TJ) proteins causes blood-spinal cord barrier (BSCB) disruption by increasing the BSCB permeability. Neutrophils infiltration is one of the characteristic features of SCI. Matrix Metallopeptidase 8 (MMP-8) or neutrophil collagenase is secreted from neutrophils and mainly acts on ECM macromolecules and is considered as the most important during inflammation, healing, and physiological processes. The role of MMP-8 is not fully established in SCI. Herein, we characterized the role of MMP-8 in the pathology of SCI in rats. **Methods: -** SCI was induced to female Sprague-Dawley rats (200-220 g body weight) after moderate static compression (35 g for 5 min) at T10 spinal cord level. Furthermore, to assess the role of MMP-8 in SCI pathology, we utilized five different approaches, i) (3R)-(+)-[2-(4-Methoxybenzenesulfonyl)-1,2,3,4-tetrahydroisoquinoline-3-hydroxamate] a specific MMP-8 specific inhibitor (MMP-8I) ii) sivelestat, an inhibitor of neutrophil elastase, iii) hydroxycarbamide which bind with zinc ion in MMP-8 complex, iv) zinc chloride which cause oxidative burst and v) N-Acetyl-L-cysteine, to either inhibit MMP-8 activity directly or to modulate neutrophils, a major source of MMP-8. **Results: -** MMP-8 was up-regulated during one and three days after SCI. Moreover, in correlation with increased MMP-8; proinflammatory cytokines (TNF-α, IL-6), microglial markers (Iba-1, Mac-1), heme oxygenase-1 and neutrophil elastase was also increased significantly after SCI. The level of TJ proteins (occludin and zonula occludens-1) were decreased suggesting the disruption of BSCB after SCI. Amusingly, MMP-8 inhibition decreases the proinflammatory cytokines (TNF-α, IL-6) and increases the expression of tight junction proteins after SCI. **Conclusion: -** MMP-8 upregulation during initial phase might be directly linked to inflammatory phase during SCI. Moreover, results suggest that inhibition of MMP-8 prevents disruption of BSCB, provides neuroprotection and can be considered as a therapeutic target in SCI.

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Poster

612. Molecular Therapeutic Strategies of Spinal Cord Injury

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Program#/Poster#: 612.04/FF9

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH NINDS NS047567

Title: Effects of zolmitriptan and NMDA on the firing characteristics of bursting deep dorsal horn neurons following chronic spinal transection

Authors: *T. THAWEERATTANASINP[1], C. J. HECKMAN[2], V. M. TYSSELING[3]; [1]Physiol., Northwestern University, Feinberg Sch. of Medici, Chicago, IL; [2]Physiology, Physical Med. and Rehabilitation, Physical Therapy and Human Movement Sci., [3]Physiology, Physical Therapy and Human Movement Sci., Northwestern University, Feinberg Sch. of Med., Chicago, IL

Abstract: Loss of descending serotonin (5-HT) to the spinal cord contributes to muscle spasms at the chronic stage of spinal cord injury (SCI). At this stage, hyperexcitable motoneurons receive long excitatory postsynaptic potentials (EPSPs) which, in turn, activate their persistent inward currents (PICs) to drive muscle spasms. Deep dorsal horn (DDH) neurons with bursting behavior could be involved in triggering the long EPSPs due to their loss of inhibition in the chronically 5-HT-deprived spinal cord. In the previous study, we found bursting DDH neurons among other types of DDH neurons, i.e. single-spiking and spontaneously tonic-firing neurons, following acute spinal transection at the sacral edge of the lumbar enlargement (i.e. between L6 and S1 spinal segments) in adult mice. Only the bursting DDH neurons showed significant changes in their firing characteristics during administration of the 5-$HT_{1B/1D}$ receptor agonist zolmitriptan and NMDA. Zolmitriptan can suppress the bursting DDH neurons, while NMDA moderately facilitates them. Here in the present study, we further investigate the firing characteristics of the bursting DDH neurons following chronic spinal transection at T10 vertebral level in adult mice during administration of zolmitriptan and NMDA, using the *in vitro* sacral cord preparation and extracellular electrophysiology. Following the 10-week period of transection recovery, zolmitriptan does suppress the bursting DDH neurons by significantly reducing their evoked spikes and burst duration and delaying their first-spike latency. However, NMDA has only a small effect on the bursting DDH neurons by enhancing only their spontaneous firing rate. These results suggest that zolmitriptan may activate 5-$HT_{1B/1D}$ receptors on the bursting DDH neurons to suppress muscle spasms following chronic SCI, as this drug has been previously shown to reduce muscle spasms without affecting the intrinsic properties of spinal motoneurons. On the other hand, activation of NMDA receptors may only raise the spontaneous activity level of the bursting DDH neurons with no significant contribution to the mechanisms of muscle spasms. A future *in vivo* study in decerebrate animals is necessary to see if the bursting DDH neurons exist in the intact spinal cord.

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Poster

612. Molecular Therapeutic Strategies of Spinal Cord Injury

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Program#/Poster#: 612.05/FF10

Topic: C.09. Brain Injury and Trauma

Title: Notch signal inhibition as a promising new therapeutic approach to human ips cell derived transplantation therapy for spinal cord injury

Authors: *T. OKUBO, A. IWANAMI, J. KOHYAMA, N. NAGOSHI, G. ITAKURA, M. MATSUMOTO, M. NAKAMURA, H. OKANO;
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Abstract: [Introduction] We previously reported that transplantation of certain neural stem/progenitor cells from human iPS cells (hiPSC-NS/PCs) into the injured spinal cord resulted in tumor-like overgrowth and subsequent deterioration of motor function. Remnant immature NS/PCs must be removed or induced into more mature cell types, which may avoid tumor-like overgrowth following transplantation. Meanwhile, Notch signaling controls the neuronal differentiation of NS/PCs, and inhibition of this signaling with gamma-secretase inhibitor (GSI) induces NS/PCs to differentiate into a more mature state with limited proliferation. The purpose of the present study was to elucidate the effects of GSI on tumorigenic hiPSC-NS/PCs.
[Method] hiPSC-NS/PCs, a potentially tumor-like overgrowth cell line, were cultured with GSI. Global analyses of the gene expression profiles were performed by DNA microarray and RT-PCRs. Next, we induced contusive spinal cord injury (SCI) in mice and transplanted hiPSC-NS/PCs with or without GSI pretreatment nine days after SCI. The growth/survival and histological analyses of transplanted cells were examined with bioluminescence imaging (BLI) and immunohistochemistry. Behavioral analyses of motor function were also performed by BMS score, rota-rod test and DigiGait system.
[Result] Compared to the non-treatment (control) group, after following treatment of hiPSC-NS/PCs with GSI (GSI+ group), the gene expression profiles using DNA microarray and RT-PCR showed decreases in pluripotency and self-renewal marker-related genes. After hiPSC-NS/PCs transplantation, the photon counts of transplanted cells increased more than 10-fold from their initial values by BLI, and then showed deteriorated motor function accompanied by tumor-like overgrowth in the control group. However, in the GSI+ group, the photon counts of transplanted cells increased more slowly, but reached a plateau, then showed maintained functional recovery and reduced the tumor-like overgrowth through inhibition of cell proliferation 89 days after transplantation. In the GSI+ group, the proportion of Hu^+ neuron increased significantly, whereas the proportion of $Ki67^+$ and $Nestin^+$ cells significantly decreased compared to the control group.
[Conclusion] We confirm that GSI pretreatment of hiPSC-NS/PCs reduce the proportion of

dividing cells *in vitro*, and prevent tumor-like overgrowth of transplanted cells by inhibiting cell proliferation, resulting in safe and long-lasting functional recovery *in vivo*. Pretreatment of hiPSC-NS/PCs with GSI prior to transplantation can improve their in clinical setting.

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Poster

612. Molecular Therapeutic Strategies of Spinal Cord Injury

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VGH96S6-001

V105C-028

Title: Investigation of combination of high dose chondroitinase ABC and olfactory mucosa progenitor cells transplants on the axonal repair of spinal cord injury

Authors: *H. CHENG[1,2,3,4,5], C.-H. CHENG[3,4], C.-T. LIN[3,6], M.-J. LEE[3,7], M.-J. TSAI[3], C.-J. CHEN[3], W.-C. HUANG[1,2,3,5];
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Abstract: Spinal cord injury (SCI) often results in neuronal death and demyelination. During the healing process, glial scar formed at the lesion site is one of the major barriers to axonal regeneration. Treatment of chondroitinase ABC (ChABC) has been reported to remove CSPGs glycosaminoglycan (GAG) side chains and enhance axonal regeneration. Cell transplantation therapies for spinal cord injury contributes to restoration of neuronal function by modifying the pathophysiological process after spinal cord injury. We have demonstrated that intrathecal delivery of low-dose ChABC in the acute stage of SCI promotes axonal regrowth and functional recovery. Furthermore, ChABC administrations combining olfactory mucosa progenitor cells (OMPCs) intraspinal transplantation showed better functional recovery and enhance the

consistency of stepping in acute stage of SCI rats. However, in the sub-acute or chronic SCI patients, the dense glial scar has formed and cannot be surgery or digested by intrathecal delivery of ChABC at the injury site. Hence, we have been demonstrated intraparenchymal delivery of high dose ChABC in the sub-acute stage of SCI animal models decreased the level of CSPGs, lesion extension and area, enhanced the axonal outgrowth and improved partial functional recovery. However, previous studies indicated that single ChABC treatment may not be sufficient to enhance neuronal plasticity and the inadequate survival time and rate of OMPCs in the lesion site might limit the regenerative ability. In this study, we will investigate the effects of high dose ChABC delivery combined with repeated transplantation of OMPCs in the acute and sub-acute stage of SCI in the complete spinal cord transection model. Our results showed that there was no functional recovery following a single OMPCs transplantation. Nevertheless, repeated OMPCs transplantation significantly improved functional recovery and enhanced axons outgrowth across the lesion site. In addition, Fluorogold (FG)-positive cells were clearly detected in the red nucleus of midbrain indicated that these axons grew across the lesion site and reached the distal stump of injured spinal cord. Moreover, the degree of fibrotic scarring and macrophages infiltration also decreased after repeated OMPCs transplantation. Furthermore, we evaluated the effects of high dose ChABC combined with repeated OMPCs transplantation in the sub-acute SCI. Although the functional recovery significantly improved via either treatment, but unfortunately no synergistic therapeutic effect can be detected. This study may provide valuable information to form a better therapeutic strategy than ever for SCI in the future.

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Poster

612. Molecular Therapeutic Strategies of Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: Spinal Cord Injury Research Fund

Title: Mitochondrial biogenesis as a novel therapeutic strategy for spinal cord injury

Authors: *N. E. SCHOLPA, A. NARANG, W. WANG, D. CORUM, S. TOMLINSON, R. G. SCHNELLMANN;
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Abstract: Spinal cord injury (SCI) is characterized by mitochondrial dysfunction and loss of ATP, ultimately leading to excitotoxicity, calcium overload and oxidative stress, and further exacerbating injury in a self-propagating cycle. Therefore, therapeutics targeting reestablishment of mitochondrial function through increased mitochondrial biogenesis (MB) following SCI could potentially alleviate multiple aspects of injury progression. This study examined the effect of formoterol, an FDA-approved β_2-adrenergic receptor agonist, on MB in the spinal cord and functional recovery following SCI. Female C57BL/6 mice were subject to moderate SCI using an 80 Kdyn force-controlled pneumatic impactor-induced contusion model. Formoterol (i.p.) was administered one hour post-SCI and then daily until euthanasia. Treatment with formoterol after injury induced MB in the spinal cord, as evidenced by increased mitochondrial DNA content, and increased expression of PGC-1α, the master regulator of MB, and various mitochondrial- and nuclear-DNA encoded mitochondrial genes. Three days post-SCI, the spinal cord of formoterol-treated mice exhibited less histological damage than that of vehicle-treated mice, namely decreased loss of white matter and structural preservation of the central canal and anterior median fissure. The Basso-Mouse Scale (BMS) was used to assess locomotor capability. A BMS score of 0, observed 24 h following SCI, indicates no movement of the hind limbs, while a score of 9 indicates normal function (sham controls). Seven days post-SCI, mice treated with formoterol demonstrated improved motor function compared to vehicle-treated mice (BMS = 1 vs. 0.3). Fifteen days after injury, formoterol-treated mice exhibited further improvement in motor function (BMS = 4.5 vs. 2), approximately 50% overall functional recovery. Taken together, we suggest formoterol and MB as a therapeutic avenue for the treatment of SCI.

Disclosures: **N.E. Scholpa:** None. **A. Narang:** None. **W. Wang:** None. **D. Corum:** None. **S. Tomlinson:** None. **R.G. Schnellmann:** None.

Poster

612. Molecular Therapeutic Strategies of Spinal Cord Injury

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NMSS RG4958

Mayo Clinic Center for regenerative Medicine

Title: Targeting the thrombin receptor to modulate STAT3-driven inflammatory astrogliosis and improve recovery after spinal cord injury

Authors: *H. YOON[1], M. RODULOVIC[2], I. A. SCARISBRICK[1];
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Abstract: A significant number of neurological conditions involve deregulation of thrombin activity including hemorrhagic, infectious and traumatic injuries, however little is known regarding mechanisms of action or the utility of therapeutic modulation. Thrombin is well known for its ability to cleave soluble fibrinogen releasing fibrin monomers that support hemostasis. In addition, thrombin is a high affinity agonist for Protease Activated Receptor 1 (PAR1), also referred to as the thrombin receptor. PAR1 is a seven transmembrane G-protein-coupled receptor that is activated by extracellular N-terminal cleavage. Here we critically evaluated the role of PAR1 as a regulator of the spinal cord injury microenvironment by examining cellular, molecular and neurobehavioral outcomes after experimental contusion-compression spinal cord injury (SCI) in wild type and PAR1 knockout mice. Mice with PAR1 loss-of-function displayed improved locomotor recovery after SCI and reduced signatures of inflammation and astrogliosis, including expression of glial fibrillary acidic protein, vimentin, and STAT3 signaling. SCI-associated elevations in pro-inflammatory cytokines such as IL-1β and IL-6 were also reduced in PAR1 knockout mice and co-ordinate improvements in tissue sparing were observed. Moreover, mice with PAR1 loss-of-function showed increased preservation of NeuN-positive ventral horn neurons as well as PKCγ-positive corticospinal axons. Since perilesional astrocytes were positive for both thrombin and PAR1 we investigated the possible significance of this signaling system in primary astrocyte cultures. Introduction of thrombin into astrocyte cultures resulted in increases in the production and secretion of IL-6 and activation of STAT3 signaling in a PAR1-dependent manner. In turn, IL-6-stimulated astrocytes increased expression of both PAR1 and thrombin pointing to a model in which PAR1 activation contributes to increased astrogliosis by feedforward- and feedback-signaling dynamics. These results suggest that PAR1 sits at the interface of the proteolytic microenvironment and cellular responses that contribute to astrogliosis, pro-inflammatory events and neurodegeneration accompanying traumatic SCI. Given the significant improvements in neurobehavioral outcomes observed in mice with PAR1 loss-of-function, these studies suggest that PAR1 may serve as a useful target to modulate astrogliosis, reduce neural injury and promote an environment favorable to repair and recovery of function after spinal cord trauma.

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

Poster

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Topic: C.09. Brain Injury and Trauma

Support: Swedish Research Council Grant 2014-2306, Medicine and Health

Title: Trimethylene carbonate-caprolactone conduit with poly-p-dioxanone microfilaments to promote regeneration after chronic spinal cord injury

Authors: L. N. NOVIKOVA[1], P. J. KINGHAM[1], A. ULLRICH[2], S. OBERHOFFNER[2], M. RENARDY[3], M. DOSER[2], E. MÜLLER[3], M. WIBERG[1], *L. N. NOVIKOV[1];
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Abstract: The majority of patients with chronic spinal cord injury (SCI) have a cystic cavity surrounded by a thick fibrotic and astrocytic scar and complete loss of function below the trauma site. In these patients and in contrast to acute SCI, transplantation of bridging materials to re-connect divided spinal cord segments can be performed without interfering with spontaneous recovery. In this study we evaluated the effects of biodegradable conduits with guidance microfilaments and Schwann cells on axonal regeneration after chronic spinal cord injury in adult rats. The conduits, with an inner diameter of 1100-1200 μm and a wall thickness of 100-200 μm, were fabricated from trimethylene carbonate and ε-caprolactone (TCC) using a wet phase inversion technique. The conduit wall consisted of an inner and an outer "skin" layer with nanoporous structure and small cavities to reduce adverse macrophage infiltration. The microfilaments with longitudinal grooves were produced from poly-p-dioxanone (PDO). Cultured Schwann cells were suspended in diluted fibrin matrix and added to the conduit immediately before transplantation. In vitro studies demonstrated that Schwann cells and adult rat dorsal root ganglia neurons grew on the PDO filaments and that the materials did not induce activation of cultured astrocytes. For in vivo experiments, a spinal cord hemisection was performed at the C3-C4 cervical level. At 2 and 5 months after surgery, the injury site was exposed, the scar tissue was resected and a 2 mm long cavity was created on one side of the cord. The conduits were transplanted into the cavity and animals were allowed to survive for an additional 2 months. Animals with conduits transplanted into acute SCI served as baseline controls. Transplantation of TCC-PDO conduit alone into acute SCI supported ingrowth of numerous sensory CGRP axons and motor 5HT axons, and migration of host Schwann cells and macrophages. Regenerating axons grew along the PDO filaments but were separated from the material surface by migrating cells. When conduits were transplanted into animals with chronic SCI, only sensory CGRP axons were found in the conduit and regenerating fibres were often associated with host Schwann cells. Addition of cultured adult Schwann cells to the conduit stimulated ingrowth of both the sensory CGRP axons and motor 5HT axons into the conduit. Both host Schwann cells and GFP-labelled transplanted Schwann cells were found in close association with regenerating fibres. The conduit had no effect on the reaction of astrocytes and microglia/macrophages in the host tissue. These results suggest that a TCC-PDO conduit with Schwann cells can promote axonal regeneration after chronic SCI.

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Poster

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Topic: C.09. Brain Injury and Trauma

Support: NIH Grant 1R01NS081040-01

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Title: BET proteins modulate the inflammatory response after spinal cord injury

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[1]Neurosurg., [2]Psychiatry and Behavioral Sci., Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: Inflammation after spinal cord injury (SCI) is a major source of damage that expands the primary lesion site. Anti-inflammatory therapeutics have shown promise in pre-clinical studies, suggesting that inflammation is an important therapeutic target in acute SCI. Bromodomain and extraterminal domain-containing proteins (BETs) are epigenetic adaptor proteins important for inflammatory transcription. Inhibition of BETs decreases expression of pro-inflammatory signaling molecules *in vitro* and attenuates inflammation in multiple animal models of inflammatory disease. Because BETs are important mediators of inflammation in many diverse mammalian tissues, I hypothesize that BET proteins modulate the inflammatory response after SCI. Using *in vitro* assays, I have demonstrated that BET inhibition attenuates inflammatory signaling by both mouse primary astrocytes and macrophages. Furthermore, BET inhibition in experimental SCI results in decreased expression of pro-inflammatory cytokines, decreased cyclin expression, and mild improvements in locomotor recovery. These results suggest a role for BETs in promoting damaging inflammation after SCI. The study of BETs after SCI is especially timely given the recent development of selective BET bromodomain inhibitors and their use in clinical trials. These studies will contribute to the understanding of epigenetic modulation of inflammation and of BETs as therapeutic targets after SCI.

Disclosures: **M.D. Trojanowsky:** None. **N.G. Ayad:** A. Employment/Salary (full or part-time): University of Miami Miller School of Medicine. **J.K. Lee:** A. Employment/Salary (full or part-time): University of Miami Miller School of Medicine.

Poster

612. Molecular Therapeutic Strategies of Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: UCLA HSSEAS Laboratory Start-Up Funds

University of California Faculty Career Development Award

Title: Gene delivery from injectable, hyaluronic acid-based hydrogels with *In situ*-forming macropores for spinal cord regeneration

Authors: *A. A. EHSANIPOUR, T. ABOUFADEL, P. COX, C. WALTHERS, W. XIAO, S. K. SEIDLITS;
Bioengineering, UCLA, Los Angeles, CA

Abstract: Spinal cord injury (SCI) is the second largest contributor to paralysis worldwide and inflicts up to 80 million people per year. Regeneration is inhibited by the secondary injury cascade, characterized by death or injury of neural and glial cells and the formation of glial scar tissue which may act as a barrier to infiltrating axons. Tissue engineering approaches including delivery of hydrogels, gene vectors, cells, or signals to the injured spinal cord have been investigated to mitigate glial scar formation and loss of function after SCI while providing scaffolds capable of integrating with neural tissue. Injectable treatments are ideal candidates as they prevent excision of potentially spared tissue or overly invasive procedures, however previous injectable therapies have had limited tissue integration, cell infiltration, and transfection by lentivirus encoding for regenerative transgenes. We attempt to improve these processes by incorporating macroporosity into an injectable SCI therapy.
Our unique injectable, macroporous hydrogel platform is based on hyaluronic acid (HA), a common matrix component in the central nervous system (CNS), which may reduce the immune response and glial scar formation after SCI, and fibrin, which can support ingrowth of cells and axons after SCI. By varying polymer concentration, these hydrogels can be tuned to mimic the mechanical properties of brain and spinal cord tissues, ranging from 100Pa to 10kPa. In a preliminary trial, soft HA hydrogels (1kPa) carrying neural progenitor cells (NPCs) were able to successfully form *in situ* in the mouse brain. Treated mice (HA+NPC) survived comparably to

mice treated with NPCs only and showed no adverse reactions to HA gels.
To achieve macroporosity, we encapsulated sacrificial, fibrin microparticles (FMP), which, after degradation within the bulk HA hydrogel, produce macroporous systems that improve nutrient transport, cell infiltration, and viral transduction. Hydrogels with increasing FMP concentrations (0, 33, 50, and 75% of scaffold volume) were delivered to the mammary fat pad of female C57/BL mice. The incorporation of FMPs increased cell infiltration into scaffolds and improved transduction efficiency by firefly-luciferase encoding lentiviral vectors up to 25-fold. This strategy will be translated to an SCI model to deliver lentivirus encoding for regenerative growth factors to evaluate their potential to promote functional recovery. This approach presents an injectable, macroporous, hydrogel platform which is compatible with delivery to the CNS, capable of improving cell infiltration and viral transduction, and holds potential to support recovery after SCI.

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Poster

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: NSF EEC-1028725

Title: Flexible fibers for optoelectronic probing of spinal cord circuits

Authors: ***C. LU**, S. PARK, U. FRORIEP, A. DERRY, J. SELVIDGE, Y. FINK, P. ANIKEEVA;
DMSE, MIT, Cambridge, MA

Abstract: While the majority of the neural engineering efforts of the past decade have focused on interfaces with the brain, fundamental understanding of the spinal cord neural dynamics remains limited by the tools capable of recording and modulation in this organ. Fueled by advances in optogenetics, optoelectronic probes have recently enabled cell-specific neural stimulation compatible with concomitant recording of neural activity. The mechanics of the spinal cord, however, remain difficult to match with hard materials traditionally used in optoelectronics.
Here we address the elastic modulus mismatch by designing flexible multifunctional neural probes capable of conforming to the spinal cord geometry and mechanical properties, while

providing optical stimulation and neural recording.
We mimicked the fibrous and flexible morphology of the spinal cord and fabricated all-polymer fibers that consist of a polycarbonate (PC) and cyclic olefin copolymer (COC) waveguide and carbon-polyethylene (CPE) composite electrodes. The conductive polymer composite electrodes exhibited tip impedance 1-3 MΩ suitable to record local field potentials. To further reduce the impedance, we have fabricated device by dip coating PC/COC waveguides (~100 μm in diameter) with a 1 μm thick layer silver nanowires (AgNWs). The devices were then encapsulated with a layer of poly(dimethylsiloxane) (PDMS) to protect the AgNW mesh from degradation, while maintaining high flexibility. Exposed AgNW mesh ring-electrodes possessed low impedance (~200 kOhm) and were capable of recording isolated action potentials in the mouse spinal cord with high signal-to-noise ratio (SNR 7±1) and low noise level (10 ±3 μV). We also applied the dip coating approach to fibers made of cyclic olefin copolymer elastomers (COCE). COCE is a rubbery material which allows COCE/AgNW fiber electrodes to maintain their conductivity at 100 % extension strain, and could be deformed repeatedly without hysteresis at 20 % strain.
The mechanical properties of the integrated fiber probes matched those of biological tissues, which is difficult to achieve with traditional metallic and semiconducting electrodes. We demonstrated the utility our devices for recording and optical stimulation in the spinal cord of transgenic mice expressing the light sensitive protein channelrhodopsin 2 (ChR2). Furthermore, we found that optical stimulation of the spinal cord with the polymer fiber probes induces on-demand limb movements. Finally, we illustrated that the modest dimensions and high flexibility of our devices permit chronic implantation into mouse spinal cords with minimal damage to the neural tissue.

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Poster

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH Grant NS082463

Barrow Neurological Foundation

Title: Changes in the afterhyperpolarizations of rat hindlimb motoneurons following incomplete spinal cord injury and exercise

Authors: *V. V. TURKIN, D. O'NEILL, T. M. HAMM;
Barrow Neurolog Inst., Phoenix, AZ

Abstract: The afterhyperpolarization (AHP) is a critical determinant of motoneuron (MN) discharge properties. We compared the amplitudes and durations of hindlimb MN AHPs in normal rats and rats with incomplete spinal cord injuries (iSCI) to determine whether such injuries alter the distribution of AHP amplitudes and durations. Rats received sham operations (sham group) or moderate contusion injuries (170 kdynes) at T9. Injured animals were randomly assigned to standard caging (SCI-standard group) or cages with exercise wheels (SCI-exercise group) 1 week after injury to determine whether exercise affected AHP properties following iSCI. AHPs were recorded primarily in tibial MNs with ketamine-xylazine anesthesia in terminal experiments 8-10 weeks following sham surgery or injury. AHP amplitudes varied between groups. Mean AHP amplitudes were greater in SCI-exercise than in SCI-standard animals. Half-decay times did not differ between groups. Amplitude and half-decay time were both correlated with input resistance (R_N) in all three groups. The largest AHP amplitudes occurred in SCI-exercise rats throughout the range of R_N, particularly at small and intermediate R_N values, indicating that AHP amplitudes increased with exercise following iSCI in larger MNs. In all groups, AHP amplitude was correlated with half-decay time, but a subset of MNs had small AHP amplitudes (< 1.5 mV) and long half-decay times (> 15 msec). We examined MNs with AHP half-decay times greater than 15 msec, associated with the presence of SK3 channels (Deardorff et al., 2013), and compared those with small (< 1.5 mV) and large (> 1.5 mV) AHP amplitudes. MNs having small AHP amplitudes had smaller input resistance values (2.2 vs. 3.5 MOhms) and larger rheobase currents (8.2 vs. 3.9nA) than those with large AHP amplitudes. MNs from sham, SCI-standard and SCI-exercise rats were not distributed evenly between small- and large-AHP amplitude subsets. SCI-standard rats had more MNs than expected in the small-amplitude subset, while sham and SCI-exercise rats had more than expected in the large-amplitude subset. In the large-amplitude subset, cells are found at all R_N values in the sham and SCI-exercise groups, but only at larger R_N values for SCI-standard rats. In the small-amplitude subset, only small R_N values are found for sham animals and most SCI-exercise rats. R_N values for SCI-standard in this subset are more broadly distributed but smaller than the values in the large-amplitude subset. Thus, long-duration, small-amplitude AHPs occur in some large MNs. The occurrence and distribution of these atypical AHPs change following iSCI but are largely restored by exercise.

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Poster

612. Molecular Therapeutic Strategies of Spinal Cord Injury

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Topic: E.09. Spinal Cord Injury and Plasticity

Title: Effects of iTBS on motor evoked potentials and H-reflexes in chronic incomplete spinal cord injury: a pilot study

Authors: H. J. FASSETT, C. V. TURCO, T. LULIC, J. EL SAYES, *A. J. NELSON;
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Abstract: Objectives: The use of intermittent theta burst transcranial magnetic stimulation (TBS) induces short-term changes in the excitability of human primary motor cortex (M1). In a placebo-controlled pilot study, we examined the effects of iTBS on motor evoked potentials (MEPs) recorded from the flexor carpi radialis (FCR) muscle in individuals with chronic cervical spinal cord injury (SCI). This pilot study aimed to examine whether the effects of iTBS over either M1 or adjacent primary somatosensory cortex (SI) influences corticospinal excitability in SCI. **Methods**: Participants (N=5) with incomplete cervical spinal cord injury (ASIA B, C or D) with an injury occurring > 1 year were recruited. ITBS involved 600 biphasic pulses at 30 Hz using a stimulator intensity of 80% AMT over the hotspot for FCR. All participants attended 3 separate sessions wherein one of the following was delivered in a pseudo-randomized order 1) iTBS over M1 2) iTBS over SI (~2cm posterior to the FCR hotspot) and 3) sham iTBS over M1. For all sessions, iTBS was delivered to the hemisphere contralateral to the least affected FCR in each participant. Dependent measures were acquired before and immediately following iTBS and included recruitment curves for MEPs and H-reflexes. MEP recruitment curves were recorded from FCR ranging from 10 - 160% RMT. H-reflexes recruitment curves were elicited using a constant current stimulator in steps of 0.5 mA until no further increase was observed. **Results:** There were no significant changes in the amplitude or slope of the recruitment curves for MEPs or H-reflexes for any of the interventions. **Discussion:** In this pilot study demonstrated that iTBS delivered to either M1 or SI do not result in short-term changes in corticospinal excitability in the chronic SCI population studied.

Disclosures: H.J. Fassett: None. **C.V. Turco:** None. **T. Lulic:** None. **J. El Sayes:** None. **A.J. Nelson:** None.

Poster

612. Molecular Therapeutic Strategies of Spinal Cord Injury

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: Ludvig & Sara Elsass foundation

Title: Botulinum toxin injection in the triceps surae muscle causes plastic adaptations in rat spinal cord circuitries.

Authors: *J. B. NIELSEN[1], J. PINGEL[2], N. D. CHRISTIANSEN[2], D. B. JENSEN[2], H. HULTBORN[2];
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Abstract: Botulinum toxin (Btx) is used in children with cerebral palsy and other neurological patients to diminish spasticity and reduce the risk of development of contractures. Btx prevents release of acethylcholine at the motor end plate and thereby induces muscle paresis. We hypothesized that this may lead to adaptive plastic changes in spinal cord motoneurones and interneuronal circuitries. We consequently investigated changes in spinal cord circuitries 1-8 weeks following Btx injection in the triceps surae muscle in rats.
A total of 58 rats were injected with Botulinum toxin (Btx; 6IU) in the left triceps surae muscle. This resulted in a decrease of muscle weight and maximal muscle force to 20 % of the intact non-injected side, lasting at least 8 weeks. Two weeks after Btx injection, stretch of the injected triceps surae muscle elicited reflex activity with larger amplitude relative to the maximal muscle force than on the non-injected side, and was elicited by lower velocity and amplitude of stretch. Increased central gain of the monosynaptic stretch reflex pathway was confirmed by the demonstration of larger monosynaptic reflexes recorded from the left (injected) than the right (non-injected) L5 ventral root following stimulation of the respective dorsal roots two weeks after Btx injection. Preliminary data suggest that a similar increase in response at the Btx-injected side to stimulation of descending motor tracts may occur, suggesting that increased excitability of spinal motoneurones may at least partly explain the increased reflexes.
The data demonstrate that muscle paresis induced by Btx injection is accompanied by plastic adaptations in the central stretch reflex circuitry, which counteract the intended antispastic effect of Btx.

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Poster

612. Molecular Therapeutic Strategies of Spinal Cord Injury

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: Leona M. and Harry B. Helmsley Chartiable Trust

Kosair Charities

Title: Functional neurophysiological assessment of volitional motor control following pediatric spinal cord injury

Authors: *D. A. ATKINSON[1,3], S. J. HARKEMA[2,3], S. A. TRIMBLE[3,4], L. X. MENDEZ[3,4,2], A. L. BEHRMAN[2,3,4];
[1]Dept. of Anatom. Sci. and Neurobio., [2]Dept. of Neurosurg., Univ. of Louisville, Louisville, KY; [3]Univ. of Louisville, Kentucky Spinal Cord Injury Res. Ctr., Louisville, KY; [4]Kosair Charities Ctr. for Pediatric Neurorecovery, Louisville, KY

Abstract: Measurement of spinal cord injury (SCI) severity and residual motor capacity in young children presents unique difficulties; the International Society for Neurological Classification of Spinal Cord Injury's American Spinal Injury Association (ASIA) Impairment Scale is not valid for children younger than six years old (Mulcahey et al. , 2011). We adapted the functional neurophysiological assessment (Li et al. , 2012) a standardized, quantitative assessment of volitional motor function, for use in children with SCI. The assessment consists of repeated attempts of standardized single- and multi-joint movements as well as passive cycling and reflex testing, while recording electromyographic activity from 11 trunk and leg muscles bilaterally. We performed this assessment on 13 typically developing children between the ages of 3 and 9 (6.64 ± 2.48) years old, and in 12 children with SCI, ages 2-10 (6.65 ± 2.52) years old. All SCI participants were injured between birth and 6 years of age (2.86 ± 2.11), and the average time since injury was 3.79 ± 2.68 years. All non-injured children were able to perform isolated movement attempts at the knee and ankle. In spinal cord injured children, isolated single joint movement in the knee was observed in one participant, although bilateral knee extensor activity was recorded. We did not observe isolated ankle movement in any spinal cord injured children. Multi-joint and/or bilateral muscle activation was observed during isolated movement attempts in 5 children, while the remaining 7 did not exhibit any muscle activation during lower extremity movement trials. Of those who did not demonstrate the ability to generate muscle activity during lower extremity movement trials, 3 exhibited delayed onset, bilateral activity in all muscles during trunk flexion trials. This pattern of activation has been described in adults (Dimitrijevic et al. , 1984), and is thought to indicate sub-clinical descending influence of supraspinal structures on spinal motor circuitry below the level of lesion. The pediatric functional neurophysiological assessment allows objective measurement and increased sensitivity in the identification and characterization of volitional movement capacity and subclinical descending motor excitation in children as young as 2 years old. Combined with electromyographic assessment of activation during tasks involving the integration of descending and peripheral inputs (such as standing and stepping), this assessment can provide a more comprehensive and objective characterization of motor capacity in children with SCI than currently available measurement tools.

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Poster

612. Molecular Therapeutic Strategies of Spinal Cord Injury

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: MOST 104-2314-B-182-007-MY3

Title: The modulation of disynaptic reciprocal inhibition in people with spinal cord injury

Authors: *Y.-J. CHANG[1], J.-M. CHANG[1], M.-P. LIN[1], M.-D. YU[1], M.-J. HSU[2,3], A. M. WONG[1,4];
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Abstract: Spinal circuitry was found to adapt after spinal cord injury. Loss of post activation depression and reciprocal inhibition was related to the spasticity after spinal cord injury (SCI). Studies showed that antagonist muscle contractions could increase the strength of disynaptic reciprocal inhibition in healthy people. The purpose was to evaluate if the electrical stimulation (ES) elicited reciprocal contractions of agonist and antagonist musclescould modulate the disynaptic inhibition in indivicuals with SCI. Five individuals with acute SCI and seven individuals with chronic SCI were recruited. The electrical stimulation were applied on tibia nerve and common peroneal nerve to elicit H reflex and conditioning stimulation. Disynaptic inhibition strength was quantified by the normalized difference between the control and tested H reflexes. The tests were performed before and after 30 minutes of ES elicited reciprocal contractions of dorsiflexor and plantarflexor. The results showed that the disynaptic inhibition was absent in chronic SCI subjects. The disynaptic inhibition strength increased after 30 minutes of contraction in acute SCI but not in chronic SCI subjects. This study concluded that the short term reciprocal types of muscle contraction could modulate spinal circuitry in individuals with acute SCI whose spinal circuitry was not fully adapted. Further studies are needed for individuals whose spinal circuitry has been already adapted.

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Poster

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: Craig H Neilsen Foundation

Department of Veterans Affeirs Merit Award

NYS SCIRB

Title: Viral vector mediated neutralization of NG2 proteoglycan (AAV-NG2Ab) combined with delivery of neurotrophin NT-3 (AAV-NT3) improves transmission, locomotion and urinary tract function after incomplete spinal cord injury in adult rats.

Authors: *V. L. ARVANIAN[1,2], H. PETROSYAN[2], V. ALESSI[2], N. PHAGU[2], J. LEVINE[2], W. F. I. COLLINS[2];
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Abstract: We recently demonstrated beneficial effects of neutralization of the NG2 proteoglycan using monoclonal antibodies delivered intrathecally via osmotic mini-pumps after spinal cord injury (SCI). Currently we have developed a novel gene therapy tool for prolonged and clinically-relevant delivery of a recombinant single chain variable fragment (scFv) anti-NG2 antibody: AAV-rh.10 serotype expressing scFv-NG2. Here we examined effects of AAV-NG2Ab alone or in combination with AAV-NT3 in adult rats with thoracic T10 contusion injuries. Immediately after injury, animals received intraspinal injections of either control AAV-GFP, AAV-NG2Ab, AAV-NT3 or AAV-NG2Ab plus AAV-NT3. Two sets of experiments were performed. 1st: Locomotor function was assessed using a battery of behavioral tests (BBB, Ladder, Narrowing beam and Catwalk) for 9 weeks post-injury followed by electrophysiological evaluation of synaptic transmission through spared axons spanning injury epicenter to individual lumbar motoneurons and to hindlimb muscles. 2nd: Lower urinary tract (LUT) function was assessed during the survival period using metabolic chambers to collect and quantify overnight urine production and after 6 weeks during terminal cystometry electrophysiology recordings with simultaneous acquisition of external urethral sphincter (EUS) EMG activity and bladder pressure. Analysis included bladder pressure threshold for and peak bladder pressure during contractions, inter-micturition interval, void volume, residual volume, duration of EUS bursting, and amplitude and duration of EUS activity. BDA tracing was used in both sets of experiments to evaluate any effects of treatment on anatomical plasticity. We found that AAV-NT3 treatment group did not show significant improvements in the first set of experiments and thus was not used in the second set. Both the AAV-NG2Ab and AAV-NG2Ab combined with AAV-NT3

treatment groups demonstrated significant improvements in all behavioral tests compared to other SCI groups. In-vivo intracellular recordings revealed an improved transmission through axons spanning the injury epicenter. Best improvements of both transmission and motor function were seen in animals that received treatment with AAV-NG2Ab combined with AAV-NT3. This combinational treatment improved LUT function as well. Improvements were evident in increased number of voids, smaller mass per void and decreased time between voids. Consistent with these results, cystometry electrophysiology demonstrated the following improvements: reduced number of non-voiding contractions, smaller residual volume, and apparent EUS bursting activity during contractions

Disclosures: V.L. Arvanian: None. **H. Petrosyan:** None. **V. Alessi:** None. **N. Phagu:** None. **J. Levine:** None. **W.F.I. Collins:** None.

Poster

612. Molecular Therapeutic Strategies of Spinal Cord Injury

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 612.19/GG6

Topic: E.09. Spinal Cord Injury and Plasticity

Support: FORB

Title: MicroRNA expression profiling in a mouse spinal cord injury model

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Abstract: Spinal cord injury (SCI) is a devastating condition resulting in permanent and irreversible deficits. Despite neurons try to regenerate, their attempts fail due to several dysfunctions. Several studies have already revealed a bidirectional alteration of microRNA following SCI, with some described to be detrimental and some protective. However there is still little evidence of specific axon regrowth microRNA clusters. The challenging goal of this study is focused on evaluating microRNA expression changes in sensorimotor cortex and more specifically in corticospinal motoneurons (CSMN), whose axons are severed by SCI. We intend to select and manipulate microRNAs involved in the regeneration process in order to restore their physiological functions and promote axon regrowth. Complete transection of the spinal cord was induced at the C7 level in 15 postnatal C57BL/6j male mice at 12h after injury (3 WT vs 3 SCI)

and in adult mice at 12h, 3 days and 7 days post injury (3 WT vs 3 SCI per each group). The sensorimotor cortex was dissected, collected for RNA extraction and sequenced for a microRNA profile to evaluate expression changes among the groups. Another set of animals was employed to analyze cortical astrogliosis (GFAP), microglia activation (IBA1) and degenerating neurons (Fluoro-Jade staining). MicroRNAs mainly implicated in neuroplasticity, axon regrowth (miR-19b-3p, miR-381-3p and miR7b-3p) and inflammation (miR-127-5p) resulted dysregulated in the injured groups. The inflammatory microRNA expression seems to follow the glial cells reaction evolving from very low level (12h) to a peak (3 days) and a final decrease at 7 days post injury. Based on these preliminary results, in order to achieve the specific CSMN microRNA profiling, motoneurons will be retrogradely labeled by fluorescent beads and isolated by FACS (Fluorescence-activated cell sorting) or laser capture microdissection. These data will allow us to better select only those small RNAs implicated in the regenerative process and use them as a powerful tool to promote axon regrowth.

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Poster

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Support: DoD W81XWH-14-1-0069

NIH R01NS072171

Title: Transcranial magnetic stimulation induces somatosensory plasticity and improves behavioral outcome following spinal cord injury in a rat model

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Abstract: Spinal cord injury (SCI) is the leading cause of disability causing partial or complete damage to sensory and motor pathways, thereby altering neural circuits. Evidence suggests that within minutes after SCI, decreases in spontaneous neuronal activity are observed in cortical areas that correspond to the injured as well as to the non-injured limbs. Moreover, these decreases were correlated with poor recovery. Thus, we hypothesized that an intervention to

attenuate the decreases in neuronal activity applied immediately post-SCI would accelerate neurorehabilitation. Transcranial magnetic stimulation (TMS) is a non-invasive method to induce neuronal excitation and plasticity beyond the stimulation period. We have recently demonstrated that following traumatic brain injury in rats TMS has rescued neuronal activity and led to improvement in behavioral tests (Lu et al., *Scientific Reports*, 2015). Therefore, we have determined if TMS can improve functional outcome post-SCI. SCI was induced at segment T7 in adult rats. We tested sensory and motor functions in three groups: SCI rats that received TMS 10 min after the procedure (immediate-TMS); SCI rats that received TMS starting two weeks after the procedure (delayed-TMS), and SCI rats that received sham TMS (no-TMS). High-frequency (20 Hz) TMS was applied to the sensorimotor cortex via a custom built rodent coil, and was delivered for 10 min, there times a week, for a total of six weeks. Sensory responses to hind limb stimulation were assessed using functional MRI (fMRI) carried out with an 11.7-T scanner which permitted high spatial and temporal resolution. The extent of the fMRI responses to hind limb tactile stimulation were calculated in the corresponding primary somatosensory areas. Preliminary results show that the immediate-TMS group demonstrated the greatest sensory responses with 50.89 ± 8.24 and 51.22 ± 4.92 activated pixels over the right (RHL) and left hind limb (LHL), respectively; the delayed-TMS group showed moderate sensory responses with 35± 2.61 and 32.8 ± 4.04 activated pixels in RHL and LHL; finally, the no-TMS group exhibited low sensory responses with 25± 4.73 and 13 ± 1.53 activated pixels in RHL and LHL, respectively. Motor behavior was assessed by weekly grid walk test, which indicated that the immediate-TMS group had fewer number of footfall errors compared to the delayed-TMS and no-TMS groups. Together, these results reveal the efficacy of TMS in improving outcomes after SCI. We anticipate that application of TMS as a therapeutic strategy could be readily translated into the clinical setting as an alternative or adjuvant to traditional rehabilitation strategies.

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Poster

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Topic: C.09. Brain Injury and Trauma

Title: Effect of a polymer implant of PPyI after injury by complete section of spinal cord in Rhesus monkeys

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Abstract: The main problem of traumatic spinal cord injury TSCI lies in its complexity, as their pathophysiology involves mechanical damage both the primary and secondary mechanical damage. It currently do not have any effective treatment to restore neurological function lost as a result of TSCI. Therefore, our research group has conducted previous studies with polymeric implants of polypyrrole iodine PPyI, which have been shown to have a beneficial effect on some processes of the pathophysiology of TSCI by favoring the nerve tissue preservation and functional recovery in rats. In the present study the implant of PPyI effect was evaluated in Rhesus monkeys. Two females of Macaca mulatta species were used, weighing 5kg to which injury underwent complete spinal cord section CSCIS at the thoracic level 9 under anesthetic effect, and in an operating room. One of these animals received the implant of PPyI CSCIS+PPyI and the other not control. Studies magnetic resonance imaging MRI were performed before and immediately after the surgical procedure, which was repeated 1, 2 and 3 months after CSCIS and fractional anisotropy FA was determined at the times mentioned above. Clinical evaluation was performed daily including Babinski, pulpejo, plantar pressure and patellar reflexes and also measures the diameter of the upper thigh, mid thigh and calf were taken for 4 months. Subsequently, histological evaluation was performed. The results show that the animal receiving the implant of PPyI presented a smaller loss of muscle mass in paralyzed limbs and plantar reflex was more evident when compared to the control animal. In the histological evaluation animal with the implant showed the presence of neurons and better preservation of cytoarchitecture spinal cord compared to the animal that did not received the implant, consistent with that observed in the MRI, while the animal that did not receive the implant presented larger cysts and medullary tissue damage value FA pre CSCIS was 0.9 in the control monkey, which after injury decreased from 0.45 at baseline to 0.15 at the end of the track, while the value of FA in the animal receiving the implant PPYI decreased after injury to 0.3, but increased as time passed until reaching a value of 0.75. The implant of PPYI has neuroprotective effect after a CSCIS to preserve the cytoarchitecture of nerve tissue and maintain FA near baseline after a CSCIS.

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Poster

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Topic: C.09. Brain Injury and Trauma

Support: CIMO TM-15-9530

Title: Spinal paired associative stimulation with novel parameters induces plasticity at wide range of interstimulus intervals and provides clinically relevant benefits upon long-term use in spinal cord injury patients

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Abstract: Spinal paired associative stimulation (PAS) is a non-invasive technique where transcranial magnetic brain stimulation (TMS) is synchronized with electrical peripheral nerve stimulation (PNS). The aim of spinal PAS is to synchronize pre-and postsynaptic activation at the level of corticomotoneuronal synapses. It is known that single PAS session can induce transient plasticity in human corticospinal tract. Spinal PAS with commonly used parameters leads to potentiation of motor-evoked potentials (MEPs) at a limited range of interstimulus intervals (the intervals between TMS and PNS). This can limit its value as a technique for long-term treatment in neurological patients, since in these patients measurements required for exact calculation of effective interstimulus intervals might be challenging, and conductivity of the neuronal tracts might change over time. Here we report novel parameters for spinal PAS that lead to potentiation of motor-evoked potentials (MEPs) at a wide range of interstimulus intervals in healthy subjects. We also investigated whether long-term PAS was capable to induce plastic changes in spinal cord injury (SCI) patients. We present several tetraplegic and paraplegic patients in whom long-term use of PAS with these parameters induced clinically relevant improvement in upper or lower limbs, respectively. Our study is a starting point for further development of this technique for the rehabilitation of SCI patients.

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Poster

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VA Research Service Merit Review Award I01BX000873

Title: Drawing breath after cervical contusion: from functional deficits to recovery

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Abstract: Spinal cord injury can cause profound and robust alterations to respiratory function and capacity. However, the inherent plasticity of the respiratory motor system is thought to be a key reason why activity and homeostasis can be maintained after moderate injury, mitigating the functional impact of the anatomical damage. We embarked on a series of experiments to determine if a moderate high cervical contusion injury would cause a measurable deficit in respiratory motor function under 'normal' breathing conditions at acute to chronic stages post trauma. Through plethysmography and electromyography we assessed respiratory output from 4 hours to 12 weeks after a moderate C3 contusion. Through specific analytical procedures, contused animals showed significant decreases in tidal and minute volumes 6 weeks post injury. Further, the degree of change in these variables for contused animals was significantly different from controls, and robust, lasting from 3 days to 12 weeks post injury. In deeper analysis of this data we assessed the degree to which the contusion injury impacted ventilatory pattern variability. Through assessment of original and surrogate data sets, the injury-induced alterations in mutual information and sample entropy show that cervical contusion significantly and robustly decreases the variability of ventilatory pattern again from 3 days to 12 weeks post injury. The enduring deficit to the respiratory motor system caused by contusion was further confirmed through electromyography recordings. When isolated via a lesion, these contused pathways were insufficient to maintain respiratory activity at all time points post injury. Collectively these data illustrate that, counter to the prevailing literature, in our model a profound and lasting functional respiratory deficit may be measured through multiple physiological assessments at all time points

after cervical contusion injury. Further, they demonstrate that the plasticity inherent within the respiratory motor system is not sufficient to induce functional ventilatory recovery as animals progress from acute to chronic time points. However, we provide evidence to suggest that the exogenous induction of plasticity through chondroitinase induced matrix modification can act to robustly evoke recovery of respiratory function at acute and chronic time points post injury. Our continuing research holds considerable promise for a deeper understanding of spinal cord injuries and the development of novel and effectual treatment strategies to combat respiratory motor deficits regardless of the length of time that has passed following trauma.

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Poster

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Topic: C.09. Brain Injury and Trauma

Support: NIH grant NS047718

Title: Unexpected and unexplained accumulation of AAV in the pineal gland after injections into sensorimotor cortex

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Abstract: Previous reports establish that regenerative growth of CST axons and recovery of motor function are enhanced by deleting PTEN in cortical motoneurons in the sensorimotor cortex. In rats, PTEN deletion has been achieved by injecting AAV that expresses shRNA against PTEN (Lewandowski & Steward, 2015, J. Neurosci. 34, 9951-9962). The test vectors that we used also express the fluorescent marker ZsGreen (AAV/PTENshRNA-ZsGreen). In follow-up studies, we discovered that the area of expression of AAV/PTENshRNA-ZsGreen can conveniently be revealed by illuminating intact brains before sectioning with a NightSea® flashlight. In doing so, we were surprised to discover that after injections of AAV/PTENshRNA-ZsGreen into the rostral sensorimotor cortex, ZsGreen fluorescence was also prominent in the pineal gland, which is many mm distant from the injection site. The observations reported here were from a study in which adult female Fischer 344 rats received crush injuries to the spinal cord at thoracic level 9. Then, 1-2 weeks post-injury, rats received 4 injections of

AAV/PTENshRNA-ZsGreen into the rostral sensorimotor cortex. Some of the rats also received transplants of neural stem cells (NSCs) into the injury site. Rats were allowed to survive for 4 months after the AAV injections. Illumination of intact brains with a NightSea® flashlight revealed prominent ZsGreen fluorescence in the majority of rats in which the pineal remained attached to the brain. Examination of sections through the pineal gland revealed that ZsGreen fluorescence was primarily in cells near the surface of the gland, suggesting that AAV vector was selectively taken up from the CSF. The test vector used here was constructed from AAV2-9. It will be of interest to determine whether accumulation in the pineal gland is unique to certain AAV subtypes or is a general property of different AAVs. If selective uptake by the pineal gland is a general feature of AAVs, this could be a complication for AAV-based therapies. The mechanisms underlying the selective accumulation of AAV in the pineal remain to be explored.

Disclosures: **O. Steward:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Axonis. **M. Metcalfe:** None. **K.M. Yee:** None. **A. Coulibaly:** None.

Poster

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: P20MD006988

Title: Functional contexts and roles of fatty acid binding protein 4 and fatty acid binding protein 5 in rats following spinal cord injury

Authors: *J. C. LICERO CAMPBELL;
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Abstract: The pathophysiology of spinal cord injury (SCI) results from the mechanical insult and secondary phase present during injury onset and resolution respectively. The direct outcomes of mechanical injury involve death of neurons and severance of axonal connections. As a result of neuronal cell death and membrane rupture, there is marked in shift in the spinal cord lipidome which promotes differentiation of resident microglia and infiltration of peripheral inflammatory cells. Our lab and others have demonstrated the onset of a large increase in: pro-inflammatory lipids, and the ω-6: ω-3 polyunsaturated fatty acid (PUFA) ratio in injured rat spinal cord epicenters when compared to controls. Pro-inflammatory fatty acids, including ω-6, have been extensively shown to promote death of neuronal cell populations and diminish axonal

reconnection. Therefore, investigating the proteins involved in binding and shuttling of fatty acids is of great import when in search of improving locomotor and sensory recovery after SCI. Fatty acid binding proteins, particularly fatty acid binding protein 5 (FABP5) and fatty acid binding protein 4 (FABP4), have displayed great therapeutic potential in their ability to bind anti-inflammatory and pro-inflammatory lipids respectively. Our previous publications have documented the presence of FABP5 in neurons, glia, oligodendrocytes, astrocytes, and neural progenitors and its ability to promote neuronal survival and locomotor recovery through modulation of docosahexanoic acid (DHA) and eicosapentanoic acid (EPA) following spinal cord injury. Following an eight-week diet enriched with DHA, levels of mRNA and protein for FABP5 at seven days post injury showed a marked upregulation (mRNA: 556± 168% n= 7-8 rats; $p<.01$; protein: 518±195% n= 6-7 rats; $p<.05$). Furthermore, inhibition of FABP5 was shown to hinder locomotor recovery following SCI. In contrast, unpublished data from our lab has revealed an opposing role for FABP4, whose presence was only prevalent in monocytes and macrophages. Spatiotemporal studies looking at mRNA and protein levels of FABP4 in control diet rats at 1,3,7,14, and 28 days post injury, revealed a marked increase of more than 100 fold mRNA and 15 fold protein differences in injured rats compared to controls at 7 days and 28 days post injury particularly. Of note, inhibition of FABP4 using the BMS 309403 FABP4 inhibitor improved locomotor recovery scored for rats at all time points. Because of these distinct expression profiles and functional contexts we hypothesize that modulation of FABP5 and FABP4 expression after injury is essential in promoting locomotor and sensory recovery following SCI.

Disclosures: J.C. Licero Campbell: None.

Poster

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NIDA Drug Supply Program

Title: Morphine-induced cell death in a rodent model of SCI

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Abstract: Opioids are commonly used to treat pain in the acute phase of spinal cord injury (SCI). We have shown, however, that administration of morphine in this phase of SCI undermines locomotor recovery and increases pain reactivity in a rodent contusion model. These adverse effects appear to depend on kappa opioid receptor (KOR) activation. Activation of the KOR is sufficient to decrease locomotor recovery after SCI, and is necessary for the morphine-induced attenuation of function. Interestingly, we found that KORs are upregulated on microglia following SCI and morphine administration. Using flow cytometry, we showed that SCI significantly increases the number of CD45+ cells expressing KORs at 48 hrs post injury. Morphine also significantly increases the number KOR+ microglia at the lesion site. We hypothesized that morphine activates these microglial-specific KORs to increase inflammation and neurotoxicity, thereby decreasing recovery of function after SCI. To test this, the current study used immunohistochemistry to compare the temporal sequence of cell loss, with and without morphine, in the rodent model. Subjects were given a moderate spinal contusion injury or were sham controls. On the day following surgery, half of the subjects in each injury condition were treated with 10 mg of morphine (i.v.) on days 1-2, 20 mg on days 3-4, and 30 mg on days 5-7. The remaining subjects served as controls, receiving an equivalent volume of 0.9% saline across days. To assess the temporal sequence of cell loss, subjects were humanely euthanized on days 2, 4, or 8, corresponding to 24 hrs after the final dose of morphine. A 1.5 cm section of spinal cord, spanning the injury site, was collected and sectioned (20μm) for immunohistochemistry. Subsets of the tissue were stained with markers for neurons (NeuN, neurofilament), microglia (OX42, IBA1) and astrocytes (GFAP, S-100). All sections were double-labeled with Caspase-3. By Day 2 post injury, SCI significantly decreased the expression of NeuN, and increased the numbers of GFAP+ and OX-42+ cells. There was no effect of morphine on cell expression in this early phase, but by Day 4 locomotor function was reduced in the morphine-treated subjects. After 7 days of morphine administration, both neuron and glial expression were significantly decreased, as was locomotor function relative to vehicle-treated controls. In ongoing experiments, we are using immunohistochemistry to further characterize KOR expression at the site of the lesion. These data will be essential in the development of therapeutic strategies, such as adjuvant medications, that allow for the safe and effective use of opioids for pain management after SCI.

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Poster

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Title: screening of neurotrophic factors enhancing neurite outgrowth using microfluidic device

Authors: ***M. KIM**[1,2,3], J.-W. KIM[2,3], J. KIM[4], J.-H. JANG[6], J. HYUN[2,3,5];
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Abstract: Various neurotrophic factors (NTFs), which are secreted from the central and peripheral nervous systems, have crucial roles for the modulation of neural functions and enhancement of neuronal survival and regeneration. Many previous studies have administered various exogenous NTFs to neuronal cells or animal models, however the optimal dose of NTF and the selection of optimal type of NTFs for the regeneration of damaged central or peripheral nerves is still unclear.In this study, we developed a high-throughput microfluidic device which contains 32 microchambers for neuronal cell culture to detect neurite outgrowth and cell survival at 8 different concentrations of applied NTF simultaneously, and also enable the combination use of more than two types of NTFs. Our aim was to reveal which concentration and what combinations of NTFs is optimal for the regeneration of various types of neurons in the central and peripheral nervous systems. Recombinant human nerve growth factor (NGF), neurotrophin-3 (NT-3), and brain-derived neurotrophic factor (BDNF) were produced and separately applied into the microfluidic device, and cortical neurons from the brain and sensory neurons from dorsal root ganglia of rats were cultured within the device for 5 days.We found that all three NTFs were effective to increase neurite outgrowth of cortical and sensory neurons, and the optimal concentration was different according to the type of NTFs and cultured neurons. When NTFs were combined, BDNF and NT-3 was effective for the neurite outgrowth and survival of cortical neurons, and NGF alone showed better results than other combination of NTFs.We concluded that the high-throughput microfluidic device was useful to detect the optimal concentration of various NTFs for neurite outgrowth and neuronal survival, and these results will be applied to animal models of spinal cord injury and peripheral nerve injury.

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Poster

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National Natural Science Foundation of China（81171152）

Title: Genetic absence of the corticospinal tract alters spinal motoneuron regeneration in mice

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Abstract: The corticospinal tract (CST)makes direct and indirect connections with spinal motoneurons and controls limb movement, particular skilled movements. By inactivating *Celsr3* in neocortex upon *Emx1-Cre,* we established *Emx1-Cre;Celsr3*$^{f/-}$ mutants, in which corticospinal axons fail to course through the internal capsule and to innervate the spinal cord. Using this model, we tested whether absent cortical inputs affect spinal motoneuorn regeneration and survival. In adult mice, we tore off right C5-C7 motor and sensory roots, and re-implanted right C6 roots back to spinal cord. We found impaired recovery of elbow flexion, a changed motoneuron survival and less axonal regeneration and remylination in *Emx1-Cre;Celsr3*$^{f/-}$ mutants compared to the control. To further study its mechanisms, we used RNAseq to screen gene expression in the local spinal cord of control mutant mice after three days of brachial plexus avulsion, and top 200 changed genes in the mutant are enriched in immune response signaling pathway, such as more polarization of M1 and less polarization of M2 macrophages. The result is identical to the expression levels of M1 and M2 makers using immunofluorescence detection. In conclusion, we propose that the corticospinal inputs may modify the spinal microglia function and subsequently affect motoneuron regeneration and survival after brachial plexus avulsion and replantation.

Disclosures: Q. Wang: None. **L. Shi:** None. **L. Zhou:** None.

Poster

612. Molecular Therapeutic Strategies of Spinal Cord Injury

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Program#/Poster#: 612.29/HH2

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH Grant R01NS047567

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Title: Sensory transmission to motoneurons is facilitated by cold-sensing C fibres expressing TRPM8 and 5-HT_{1D} receptors: links between management of spasticity and migraines

Authors: Y. LI, A. M. LUCAS-OSMA, S. BLACK, M. J. STEPHENS, K. K. FENRICH, K. FOUAD, *D. J. BENNETT;
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Abstract: For many years we have known that serotonergic antimigraine drugs (triptans) can be used in treatment of spasticity after spinal cord injury, potently inhibiting the monosynaptic reflex (MSR) from group Ia muscle spindle afferents to motoneurons. However, triptans selectively activate 5-HT_{1D} receptors that are almost exclusively found on unmyelinated C fibres where they inhibit C fibre activity, consistent with their action in migraine treatment, but inconsistent with inhibition of the MSR. This suggests that somehow C fibres modulate MSR transmission. Intriguingly, cold-sensing C fibres (expressing TRPM8) have been implicated in migraines, and thus we specifically explored these fibres. Spinal cords of adult rats were maintained in vitro, while recording the MSR evoked on ventral roots in response to Ia afferent stimulation (1.5xT). Selective C fibre stimulation was obtained by bathing one dorsal root in low dose TTX, to block myelinated axons, but leave TTX-resistant C fibres (with Nav1.8). Electrical stimulation of these C fibres (50xT) led to a long-term facilitation of the MSR (and underlying EPSP) evoked from an adjacent dorsal root (not in TTX), indicating a facilitation of transmission in the Ia afferents. The MSR was likewise increased by cooling or pharmacological activation of cold-sensing C fibres with the TRPM8 agonist icilin. Importantly, following icilin application, electrical C fibre stimulation produced no further facilitation of the MSR, implying that cold sensing C fibres are entirely responsible for the MSR facilitation. Considering that the MSR is involved in tremor and clonus, this finding implies that cold sensing fibres can facilitate clonus. C fibre stimulation or icilin also increased dorsal root reflexes (DRR) evoked by Ia afferent stimulation, though not the primary afferent depolarization (PAD), implying a mechanism not involving PAD. Application of the 5-HT_{1D} agonist zolmitriptan (triptan) potently inhibited the MSR and DRR, and this inhibition was increased or prevented by activation or blocking cold-sensing C fibres, respectively. These results imply that afferent transmission to motoneurons is tonically facilitated by cold-sensing C fibres which in turn are gated by serotonin, and suggest a

spinal mechanism contributing to cold-induced shivering and clonus in normal and pathological states.

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Poster

613. Central Mechanisms of Pain

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Program#/Poster#: 613.01/HH3

Topic: D.02. Somatosensation: Pain

Support: NIH NS057940

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Title: Enhancing somatosensory cortical activity alleviates neuropathic pain by suppressing homeostatic hyperexcitability

Authors: ***X. JIN**[1], W. XIONG[2], X. PING[2], M. S. RIPSCH[2], G. SANTA CRUZ CHAVEZ[3], K. JIANG[4], F. WHITE[2];
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Abstract: Central sensitization and hyperexcitability of the nociceptive system is believed to be a basic neurophysiological mechanism in neuropathic pain, while suppressing such hyperexcitability by directly blocking excitation or enhancing inhibition is a major therapeutic strategy for controlling the pain. However, the current pharmacological treatments only partially reduce the pain in less than a half of the patients. Because development of neuropathic pain often involves an initial deafferentation due to a primary lesion of nociceptive pathways while deafferentation is known to cause cortical hyperexcitability through homeostatic activity regulation, we hypothesize that abnormal homeostatic activity regulation of the primary somatosensory cortex (S1) may play a role in pain development and maintenance, and that

neuropathic pain can be controlled by enhancing cortical excitatory activity. Using a transient spinal cord ischemia model of neuropathic pain in GCaMP6-expressing transgenic mice, here we used repeated *in vivo* two-photon imaging to demonstrate that spontaneous firing of layer V pyramidal neurons of the S1 was initially depressed at six hours after injury and subsequently enhanced in one to two weeks, which is consistent with homeostatic activity regulation. Specifically enhancing S1 by optogenetic stimulation both prevented the development and reduced the severity of mechanical hyperalgesia of the hindlimbs. This effect was likely medicated by excitatory drive, because transecting corpus callosum eliminated the ipsilateral, but not contralateral, analgesic effect induced by unilateral optogenetic stimulation of S1. Similar analgesic effect was achieved by pharmacologically enhancing activity by chronically releasing $GABA_A$ receptor antagonist bicuculline in the S1. Furthermore, patch clamp recordings from layer V pyramidal neurons showed that neuronal hyperexcitability in this model could be mainly attributed to increased input resistant and higher frequency of miniature excitatory postsynaptic currents, while S1 optogenetic stimulation reversed these pathological changes and reduced network hyperexcitability. We conclude that abnormal homeostatic activity regulation in the S1 is involved in the mechanism of neuropathic pain, and that stimulating cortical excitatory activity is a novel strategy for the prevention and treatment of neuropathic pain.

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Poster

613. Central Mechanisms of Pain

Location: Halls B-H

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Program#/Poster#: 613.02/HH4

Topic: D.02. Somatosensation: Pain

Title: Primary somatosensory and anterior cingulate cortex neuronal responses to mechanical stimulation are altered after spared nerve injury.

Authors: *M. A. NOTHEM, A. GRAZIANO, J. BARRETT;
Drexel Univ., Philadelphia, PA

Abstract: Chronic neuropathic pain (CNP) is a prevalent and debilitating condition that causes suffering and current treatments remain inadequate. CNP results in long-term alterations in multiple regions of the CNS that can alter processing of sensory or affective components of pain. The Primary Somatosensory cortex (S1) and Anterior Cingulate cortex (ACC) are two pain-related cortical regions that are involved in pain processing. S1 processes the location and quality of pain, while the ACC processes pain affect and motivated behavior. However, it is currently

unclear if electrophysiological properties of neurons in these cortical regions are altered during the development and maintenance of CNP. In order to identify changes in S1 and ACC neuronal activity during CNP, Sprague Dawley rats were implanted with 2 microwire arrays targeted to S1 and ACC. Neuronal activity and behavioral responses to mechanical stimulation of the hindpaws, using an electronic Von Frey device,were recorded in awake rats prior to and 3,7,14,21 and 28 days after inducing the Spared Nerve Injury (SNI) model of CNP. In ongoing experiments, we have identified parameters of neuronal activity in both S1 and ACC that are altered after SNI. Mean firing rates of ACC neurons increase as early as 3 days and maintain for up to 28 days, while mean firing rates of S1 neurons increases by day 14 and then maintains for up to 28 days. Peak responses to mechanical hindpaws stimulation increase in S1 and ACC cells within the first two weeks after SNI. The increased responses in S1 and ACC coincide with the development of hypersensitivity. Intraperitoneal injection with 30 or 100, but not 10mg/kg of gabapentin reversed mechanical hypersensitivity and mean firing rates and peak responses of ACC neurons. However, gabapentin did not have an effect on S1 neurons, despite reversing mechanical hypersensitivity. These data suggest that S1 and ACC are differentially affected by peripheral nerve injury with increases in neuronal activity occurs first in the ACC during induction and then in S1 during maintenance. Gabapentin preferentially affects ACC neurons suggesting that it may exert an effect in modulating the processing of pain affect.

Disclosures: M.A. Nothem: None. **A. Graziano:** None. **J. Barrett:** None.

Poster

613. Central Mechanisms of Pain

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Program#/Poster#: 613.03/HH5

Topic: D.02. Somatosensation: Pain

Support: NRF of Korea, MRC, 2012R1A5A2A44671346

Title: Chronic neuropathic pain is mediated by reduced metabotropic glutamate receptor 5 activity within the periaqueductal gray

Authors: *G. CHUNG[1,2], H. SHIM[1], C. KIM[1], J. LEE[1,3,4], Y. KIM[1], S. KIM[1,2];
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Abstract: Endogenous descending pain modulatory system in the brain is the final arbiter for the emergence of the chronic pain. Typically, however, majority of pain studies have focused on peripheral and spinal mechanisms, so that the mechanisms which lead to the switch of the endogenous modulatory system have not been studied. Here we demonstrate that the metabotropic glutamate receptor 5 (mGluR5) in the periaqueductal gray (PAG) is the molecular executor which determines mode of action of endogenous pain modulatory system. Using brain imaging, electrophysiological recordings and behavioral experiments, we show that the mGluR5 in the PAG (PAG-mGluR5) is persistently active in a normal condition and this activity is necessary to regulate sensory perception in an appropriate range. This mGluR5 activity is declined in chronic neuropathic pain condition, which leads to dysfunction of pain modulation. Notably, we found that the long-lasting mechanical hypersensitivity is produced in the naïve animals by single-time blockade of mGluR5 in the PAG. This suggests that the suspension of the PAG-mGluR5 is able to induce chronic failure of pain modulatory system, not merely transient dysfunction. Our findings provide new insight into how the endogenous pain modulatory system becomes dysfunctional as it contributes to chronic neuropathic pain

Disclosures: **G. Chung:** None. **H. Shim:** None. **C. Kim:** None. **J. Lee:** None. **Y. Kim:** None. **S. Kim:** None.

Poster

613. Central Mechanisms of Pain

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Grant-in-Aid for Scientific Research (C)

Title: Optogenetic dissection of the amygdala-mediated control of the descending pain modulation

Authors: Y. K. SUGIMURA[1], Z. GHASEMI[1,2], Y. TAKAHASHI[1], Y. YANAGAWA[3], R. KANEKO[3], A. M. WATABE[1], *F. KATO[1];
[1]Dept Neurosci, Jikei Univ., Tokyo, Japan; [2]Dept. of Physiology, Fac. of Med. Sci., Tarbiat Modares Univ., Tehran, Iran, Islamic Republic of; [3]Dept. of Genet. and Behavioral Neurosci., Gunma Univ. Grad. Sch. of Med., Gunma, Japan

Abstract: The central nucleus of the amygdala (CeA) is well known to play pivotal roles in various aspects of emotional learning. This nucleus is also a key structure in nociception-emotion link as it receives nociceptive information directly from the superficial layer of the dorsal horn via the lateral parabrachial nucleus (LPB). Recently, it has been shown that the CeA not only receives nociceptive information to affect emotional behaviors but also actively modulates nociceptive sensitivity. We have recently demonstrated that direct monosynaptic glutamatergic inputs from the LPB not only excite CeA neurons but also regulate CeA network signaling through robust feed-forward inhibition, suggesting that the activity of CeA network, including its output, is under plastic modulation in pain chronification (Sugimura et al., J Neurophysiol, 2016). As one of the major targets of the CeA output is the periaqueductal gray (PAG), a primary nucleus for descending pain modulation, we sought to examine how outputs from the CeA subsequently affects PAG neurons. To address this issue, we analyzed the CeA-PAG synaptic transmission by using whole-cell patch-clamp technique and optogenetics. We transfected AAV vector for channelrhodopsin-2 (ChR2)-YFP expression to the CeA in 4-5-week-old male Wistar rats or VGAT-Cre rats and prepared brain slices containing the PAG more than 5 weeks after transfection. In the ventrolateral PAG (vlPAG), fibers with YFP fluorescence were observed, and blue light stimulation evoked monosynaptic inhibitory postsynaptic current (IPSC) with very small latency fluctuation in vlPAG neurons. Moreover, IPSC amplitude was decreased by the application of a mu opioid receptor agonist, DAMGO, and this inhibitory effect of DAMGO was stronger in the formalin-induced inflammatory pain model than in the naïve rats. These results suggest that the plastic changes in CeA-PAG synaptic transmission in the sustained pain state might play a role in determining nociceptive sensitivity via the descending pain modulatory system.

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Poster

613. Central Mechanisms of Pain

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Topic: D.02. Somatosensation: Pain

Support: R01 DA 041809

R01 NS 069575

Title: Loss of diffuse noxious inhibitory control depends on kappa opioid receptor mediated signaling in a rat model of medication overuse headache

Authors: *K. M. NATION[1], P. I. HERNANDEZ[2], D. W. DODICK[3], F. PORRECA[2]; [1]GIDP in Neurosci., [2]Med. Pharmacol., Univ. of Arizona, Tucson, AZ; [3]Mayo Clin., Scottsdale, AZ

Abstract: Diffuse noxious inhibitory control (DNIC) is an endogenous, bottom-up, pain modulatory system in which one painful stimulus inhibits another painful stimulus. DNIC is tested by the concurrent application of a noxious conditioning stimulus and a noxious test stimulus to remote locations on the body. The application of the conditioning stimulus increases the pain threshold to the test stimulus in subjects with efficient DNIC. A loss of DNIC, also termed conditioned pain modulation (CPM), has been demonstrated in humans with idiopathic pain disorders, including chronic migraine and medication overuse headache (MOH). Stress is commonly reported as a trigger for migraine and dynorphin, an endogenous agonist at kappa opioid receptors (KORs), has been shown to be necessary for aversive behavioral responses to stressors; norbinaltorphimine (nor-BNI), a KOR antagonist, blocks aversive behaviors to stress. The amygdala provides an emotional component to sensory information and is part of the affective pain system. Thus, activity at KORs within the amygdala might cause a lack of resilience to stressors. Drugs used for acute treatment of migraine, including opiates, produce MOH. We have previously demonstrated that priming animals with morphine produces a loss of DNIC that can be reversed by inactivation of the rostral ventromedial medulla revealing enhanced descending facilitation. We hypothesized that the loss of DNIC in morphine-primed rats would be prevented by blockade of KOR signaling following systemic nor-BNI or by microinjection into the central nucleus of the amygdala (CeN). Male S.D. rats were given morphine or vehicle continuously for seven days. Two weeks after the end of drug treatment rats were stressed by exposure to bright lights (BLS) for one hour on two consecutive days. Two hours after the second BLS DNIC was tested by giving rats a forepaw capsaicin injection and applying the Randall-Selitto paw pressure test to the hindpaw. Nor-BNI was given by subcutaneous injection or into the right CeN one hour prior to each BLS session. Rats with previous exposure to morphine showed a loss of DNIC compared to controls. Nor-BNI given

systemically or into the right CeN prevented the loss of DNIC in morphine-treated rats. These results show that signaling at KORs has a critical role in impairment of the DNIC response in MOH. These results also show that the CeN is important in the regulation of the DNIC response in MOH rats. The CeN receives inputs from stress circuits and has outputs to descending pain modulatory centers highlighting the possibility of KOR-mediated enhanced descending facilitation as an amplifier of stress-induced hyperalgesia relevant to migraine pain.

Disclosures: K.M. Nation: None. **P.I. Hernandez:** None. **D.W. Dodick:** None. **F. Porreca:** None.

Poster

613. Central Mechanisms of Pain

Location: Halls B-H

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Program#/Poster#: 613.06/HH8

Topic: D.02. Somatosensation: Pain

Title: Morphine metabolites and their role in mediating the sexually dimorphic effects of morphine.

Authors: *H. H. DOYLE, A. Z. MURPHY;
Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: Both preclinical and clinical studies utilizing a variety of pain assays report that the effective dose for morphine is approximately 2-fold higher in females than males. Our lab has recently reported that chronic administration of morphine activates microglia and astrocytes within the periaqueductal gray (PAG), a critical site for the pain-relieving effects of morphine. Following administration, ~90% of morphine is converted into two active metabolites: morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G), each with distinct pharmacological profiles. M6G binds exclusively to μ opioid receptors (MOR) and is a potent analgesic; in contrast, M3G binds exclusively to the glial innate immune receptor, toll-like receptor 4 (TLR4). TLR4 activation by M3G initiates a neuroinflammatory response that directly opposes the analgesic effects of both morphine and M6G. In rats, M3G serum concentrations are 2-fold higher, and M3G:morphine ratio is ~5.5-fold higher in females than males. The present studies tested the hypothesis that increased M3G levels, and subsequent TLR4 activation of glia, are a primary mechanism driving the attenuated response to morphine observed in females. We report that intra-PAG administration of M6G resulted in a greater analgesic response in females relative to males, such that sex differences in analgesia were abolished. Similarly, intra-PAG administration of M3G significantly attenuated the analgesic effects of systemic morphine in

males and females. Together, these data implicate 3-glucuronide metabolites as a primary contributing factor in the attenuated response to morphine observed in females.

Disclosures: **H.H. Doyle:** None. **A.Z. Murphy:** None.

Poster

613. Central Mechanisms of Pain

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant DA027625

Title: Characterization of apomorphine-induced antinociception in the periaqueductal gray of the rat

Authors: S. M. SCHOO, *M. M. MORGAN;
Dept Psychology, Washington State Univ., Vancouver, WA

Abstract: The periaqueductal gray (PAG) is part of a descending nociceptive modulatory pathway. Opioid and dopamine neurons and receptors are found in the PAG and both contribute to PAG mediated antinociception. We have previously shown that microinjection of the dopamine receptor agonist apomorphine into the ventrolateral PAG (vlPAG) produces antinociception via a D2 receptor-mediated mechanism. The objectives of the present experiments were to determine whether the antinociceptive effects of apomorphine microinjections into the PAG are comparable in male and female rats (Experiment 1), whether opioid receptors contribute to apomorphine-induced antinociception (Experiment 2), and whether tolerance develops to repeated apomorphine microinjections (Experiment 3). Rats were implanted with a cannula aimed at the vlPAG for microinjection of the dopamine receptor agonist apomorphine (2.2, 4.6, 10, 22, & 46 μg/0.4 μl). Nociception was assessed using the hot plate test before and after microinjection of apomorphine. Experiment 1 showed that microinjection of apomorphine produced greater antinociception in male compared to female rats both in terms of potency and duration of action. Experiment 2 showed that blocking opioid receptors via naloxone (2 mg/kg, s.c.) enhanced apomorphine antinociceptive potency in male, but not female rats. Experiment 3 showed that repeated injections of apomorphine into the PAG enhanced apomorphine-mediated antinociception in both male and female rats. These data show that the antinociceptive effects of apomorphine are similar to morphine following PAG administration in producing a greater effect in male than female rats, but differ from morphine in

that antinociception is enhanced after opioid receptor blockade or following repeated administration.

Disclosures: **S.M. Schoo:** None. **M.M. Morgan:** None.

Poster

613. Central Mechanisms of Pain

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Topic: D.02. Somatosensation: Pain

Support: Grant-in-Aid for Scientific Research (C)

Title: Immunohistochemical analysis of α2δ-1 subunit of calcium channel in the locus coeruleus (LC) and the periaqueductal gray (PAG) in rats.

Authors: T. NONAKA, K. YOSHIDA, *T. YAMAMOTO;
Kumamoto Univ. Hosp., Kumamoto-shi, Japan

Abstract: Gabapentinoid, such as gabapentin and pregabalin, binds to α2δ subunit of calcium channels. Four α2δ subunit genes have been cloned and α2δ-1 subunit is thought to play an important role in pain transmission. Although gabapentinoids are widely used as an analgesic agent for neuropathic pain, the precise mechanism of gabapentinoids to produce an analgesic effect is not clear. LC, one of the nuclei of descending pain modulation system, has been reported to be the site of action of gabapentinoids. However, the location of α2δ-1 subunit in the LC has not been reported. In the present study, we examined the location of α2δ-1 subunit in nuclei of descending pain modulation system, such as LC and PAG, using immunohistochemistry technique. [Methods] The rats were anesthetized deeply and transcardially perfused with 4% paraformaldehyde. The brain was removed immediately and postfixed overnight at 4℃. Coronal sections were cut with a microtome at 50 μm. The sections were incubated with two antibodies of rabbit anti-α2δ-1 subunit (1:1000; OriGene, MD and ABGENT) for 24 hours at room temperature. The sections were then incubated for 3 hours with Donkey Anti-rabbit IgG (Alexa Fluor 488). The slices were observed and photographed under confocal laser scanning microscopy (FV 1200). [RESULTS] α2δ-1 subunit like immunoreactivity was not found in LC, but in parabrachial nucleus which was located next to LC. Some α2δ-1 subunit like immunoreactivities were found inner and outer circumference of PAG. [DISCUSSION] α2δ-1 subunit was expressed in nuclei of descending pain modulation system and descending pain modulation system may be one of the action site of gabapentinoids.

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Poster

613. Central Mechanisms of Pain

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Topic: D.02. Somatosensation: Pain

Support: Sigrid Jusélius Foundation Grant

Title: Stimulation of the secondary somatosensory cortex in neuropathic rats: contribution of the rostral ventromedial medulla to pain attenuation

Authors: ***B. SAGALAJEV**, H. VIISANEN, H. WEI, A. PERTOVAARA;
Dept. of Physiology, Fac. of Med., Univ. of Helsinki, Helsinki, Finland

Abstract: Clinically, transcranial magnetic stimulation of the secondary somatosensory cortex (S2) attenuates neuropathic pain. The mechanisms, however, of pain attenuation by S2 stimulation remain unclear. We therefore electrically stimulated the S2 in neuropathic rats and assessed the contribution to descending pain inhibition of the rostral ventromedial medulla (RVM). The model of neuropathy was spinal nerve ligation. Under light pentobarbital anesthesia, S2 stimulation prolonged the latency for paw withdrawal from heat. Pretreatment of the RVM with an agonist (8-OH-DPAT) of a 5-HT_{1A} autoreceptor, however, prevented antinociceptive action of S2 stimulation. In the RVM, we recorded presumably antinociceptive OFF cells that decrease the discharge rate in response to heat, and presumably pronociceptive ON cells that increase it. In OFF cells, S2 stimulation delayed the onset of heat responses, whereas in ON cells, it shortened the duration. Additionally, in the spinal dorsal horn, we recorded wide-dynamic-range (WDR) cells that increase the discharge rate in response to a light touch and to heat. S2 stimulation suppressed heat responses in WDR cells. Spinal administration of a selective 5-HT_{1A} receptor antagonist (WAY-100635), however, prevented suppression by S2 stimulation of heat responses in WDR cells. We conclude that, in neuropathic rats, S2 stimulation induces spinal antinociception through descending pathways extending throughout the RVM. Particularly, S2 stimulation induces analgesia-promoting discharge changes in OFF- and ON cells and activates spinally projecting serotoninergic cells acting on the spinal 5-HT_{1A} receptor.

Disclosures: **B. Sagalajev:** None. **H. Viisanen:** None. **H. Wei:** None. **A. Pertovaara:** None.

Poster

613. Central Mechanisms of Pain

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant R21-NS078619

Interim Support, Office of Research, UTHSC

Title: Inactivation of neurons in the transitional zone (TZ) in rat sensorimotor cortex reduces responsiveness of nociceptive neurons in the spinal cord to thermonoxious stimulation

Authors: *O. V. FAVOROV[1], V. PELLICIER-MORATA[2], A. DEJONGH CURRY[3], A. BRNA[1], R. S. WATERS[2];
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Abstract: Introduction: We previously reported that the transitional zone (TZ) in rat dysgranular cortex, which lies adjacent to the forepaw and hindpaw representations in somatosensory cortex (S1), contains neurons that respond to thermonoxious stimulation. When TZ is inactivated by injection of lidocaine or local cooling, responsivity to noxious stimulation is lowered in the contralateral limb, as measured by foot withdrawal in a heated water bath. In the present study, we examined whether inactivation of TZ alters the responsiveness of nociceptive neurons, recorded in the dorsal horn of the contralateral spinal cord, to thermonoxious skin stimulation. Methods: In rats maintained under isoflurane anesthesia, the skull over sensorimotor cortex was removed, and TZ was localized by microelectrode mapping of the exposed cortex using mechanical and thermonoxious stimulation. Thermonoxious stimulation was applied by lowering the hindlimb in a 50-51 degrees C heated water bath. Control water bath stimulation was set at 41 degrees C. The spinal cord was then opened in the lumbar region (L1-L3) and a microelectrode was inserted into the upper dorsal horn laminae to isolate nociresponsive neurons there and record their responses to thermoneutral and thermonoxious stimulation. TZ was then ablated and the nociceptive dorsal horn neurons were retested with the same thermoneutral and thermonoxious stimuli. Electrolytic lesions were used to mark recording sites in spinal cord. At the end of experiments, rats were perfused, sensorimotor cortex flattened and cut tangentially to reveal the barrel field and the ablated site. Cortical and spinal cord sections were stained with cytochrome oxidase. Results: (1) Thermonoxious stimulation applied to the hindlimb activated neurons in the ipsilateral dorsal spinal cord and contralateral TZ. (2) Inactivation of TZ reduced the responsiveness of nociceptive spinal cord neurons to thermonoxious stimulation. (3) Following TZ inactivation, response of nociceptive dorsal horn neurons to thermonoxious stimulation became similar to their response to thermoneutral stimulation. Conclusion: These

results strongly suggest that TZ plays an important role in pain perception through facilitatory modulation of nociceptive neurons in the spinal cord dorsal horn.

Disclosures: O.V. Favorov: None. **V. Pellicier-Morata:** None. **A. DeJongh Curry:** None. **A. Brna:** None. **R.S. Waters:** None.

Poster

613. Central Mechanisms of Pain

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Topic: D.02. Somatosensation: Pain

Support: TR01 NS081707

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T32 GM108539

Title: Divergent periaqueductal gray neuronal populations drive pruritus processing

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Abstract: Chronic itch, like chronic pain, is a major clinical problem. Despite the similarities between itch and pain, the underlying neural circuitry for itch is poorly understood, as is the mechanism by which itch is suppressed by pain. Profound analgesia occurs with stimulation of the periaqueductal gray, however the precise role of the PAG in itch is unknown. We hypothesized that specific subsets of neurons within the PAG could suppress itch and provide a neural substrate for the inverse interaction between itch and pain. To determine the role of PAG in mediating itch, we activated or inhibited PAG neurons using engineered G-protein coupled receptors (GPCRs) activated exclusively by synthetic, systemically administered small molecules (i.e., DREADD technology). Engineered excitatory (Gq) or inhibitory (Gi) GPCRs were expressed in PAG neurons via adeno-associated viral vectors. We found that chemogenetic activation of non-specific PAG neurons produced a reduction of itch, whereas inhibition of non-specific PAG neurons resulted in enhanced itch. In contrast, when only the GABAergic neurons in the PAG were targeted, activation resulted in decreased itch, conversely inhibition of PAG GABAergic neurons resulted in increased itch. When glutamatergic neurons in the PAG were selectively targeted we found that activation led to enhanced itch, while inhibition neurons

produced decreased itch. We conclude that the PAG is an essential neuronal substrate in mediating itch behaviors and is a neural control center for pruritus.

Disclosures: V.K. Samineni: None. **J.G. Grajales-Reyes:** None. **B.A. Copits:** None. **R.W. Gereau IV:** None.

Poster

613. Central Mechanisms of Pain

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 613.12/HH14

Topic: D.02. Somatosensation: Pain

Support: TR01 GM104948-03

FAER MRTG-BS-02/15/2014Ta

Title: The analgesic effect of dopamine in male and female mice

Authors: *J. PEI[1], N. E. TAYLOR[4], K. Y. VLASOV[2], J. A. GUIDERA[4], K. SOLT[4], E. N. BROWN[3];
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Abstract: Women are at substantially greater risk than men for many clinical pain conditions [1]. In addition, drugs which modulate neural dopamine (DA) represent an important novel analgesic, and yet their effects have never been studied in females. We hypothesized that female mice would be more sensitive to carrageenan-induced inflammation than males, that d-amphetamine would be as effective as morphine in treating this pain, and DA neurons in the ventral lateral periaqueductal grey (vlPAG) mediate the analgesic effect. To test this hypothesis, we used DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) to see if selective stimulation of DA neurons in the vlPAG could produce analgesia. Adult C57BL6 male and female mice received injections of carrageenan into the left hind paw to induce inflammatory pain, and thermal hyperalgesic responses were measured using the Hargreaves method. Afterwards, separate groups of mice were treated using intraperitoneal (i.p.) injections of morphine and D-amphetamine, and paw withdrawal latencies to a thermal stimulus were recorded. Adult DAT-cre mice then received bilateral injections of adeno-associated virus (AAV) carrying an excitatory DREADD (HM3Dq) in vlPAG. Mice receiving injections of AAV lacking the DREADD served as controls. After at least 4 weeks to allow for viral transfection, thermal hyperalgesia was tested in mice with carrageenan-induced inflammatory pain.

Immunohistochemistry was used to confirm viral expression and co-localization. We found that the onset of inflammatory pain was significantly faster in female BL6 mice (n=6) as they were more sensitive to the thermal stimulus in the first 3 hours following carrageenan injection than the males (3.2±0.9s vs 6.0±2.1s, $p<0.005$). However, there was no difference in paw withdrawal latencies after 6hrs (2.0±0.3s vs 2.5±0.4s). Both males and females showed significant analgesic responses with 3 mg/kg morphine (n=6) and 3 mg/kg D-Amphetamine (n=6). Analgesia was also achieved in animals with DREADD activation of vlPAG DA neurons by CNO (n=9), as they showed no significant difference in paw withdrawal latencies between treated (8.2±1.7s) and untreated paws (9.3±1.6s). DREADD viral expression and co-localization in vlPAG DA neurons was confirmed. In summary, females exhibited faster onset of inflammatory pain and greater sensitivity to analgesic medications, but were effectively treated using both systemic administration of D-amphetamine as well as selective activation of DA neurons in the vlPAG. DA modulating agents may represent a novel new treatment for pain in both males and females.
[1] Fillingim R, et al. J Pain 2009 May; 10(5): 447-485

Disclosures: **J. Pei:** None. **N.E. Taylor:** None. **K.Y. Vlasov:** None. **J.A. Guidera:** None. **K. Solt:** None. **E.N. Brown:** None.

Poster

613. Central Mechanisms of Pain

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant NS066159

NIH Grant NS093894

Title: The parabrachial complex links pain transmission to pain modulation

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Abstract: An important factor in both normal and clinically significant pain is the intrinsic pain-modulating system, which regulates nociceptive processing via projections from the brainstem to the dorsal horn. The output of this modulating system, the rostral ventromedial medulla (RVM), can facilitate or suppress nociceptive transmission at the dorsal horn by the respective action of

two distinct classes of neurons, “ON-cells” and “OFF-cells”. Both classes respond to noxious inputs: ON-cells are activated, leading to a “burst” of activity associated with behavioral responses to noxious stimulation, whilst OFF-cell firing is suppressed, producing a “pause” in any ongoing activity. However, the pathway through which noxious inputs drive changes in RVM activity is only now being defined. Anatomically, the RVM receives direct spinoreticular and trigeminal reticular inputs, as well as afferents from the parabrachial complex (PB), which is the major target of supraspinal projections from the superficial dorsal horn. The aim of this study was to test the hypothesis that noxious information is relayed to the pain-modulating neurons of the RVM via the PB, under basal conditions and in persistent inflammation. ON-cells and OFF-cells were recorded in the RVM in lightly anesthetized rats. The reflex-related ON-cell burst and OFF-cell pause were attenuated or blocked by pharmacological inactivation of the lateral PB *contralateral*, but not ipsilateral, to the noxious stimulus. By contrast, in animals subjected to chronic inflammation (CFA in one hindpaw), block of PB *ipsilateral* to the inflamed paw interfered with the ON-cell burst and OFF-cell pause elicited by normally innocuous stimulation of that paw. This implies recruitment of a normally inactive pathway during inflammation. A direct PB input to RVM was demonstrated using optogenetic methods. Inactivation of archaerhodopsin-expressing PB terminals in RVM in normal animals interfered with both the ON-cell burst and OFF-cell pause. Activation of channelrhodopsin2-expressing PB terminals in RVM was able to drive RVM neurons *in vivo*, and in an RVM adult slice preparation, evoked synaptic currents. These data show that a substantial component of the relevant nociceptive drive to RVM pain-modulating neurons is relayed through the parabrachial complex, and that RVM neurons receive direct input from PB. While the contralateral PB relays noxious input under basal conditions, the ipsilateral PB is recruited in persistent inflammation. Thus, the parabrachial nucleus, well known as an important relay for ascending nociceptive information, also accesses descending control systems through a connection with the RVM.

Disclosures: **Q. Chen:** None. **Z. Roeder:** None. **Y. Zhang:** None. **M. Li:** None. **S.L. Ingram:** None. **M.M. Heinricher:** None.

Poster

613. Central Mechanisms of Pain

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant NR013861

Title: Posterior-hypothalamic modulation of nociception for male and female rats in a neuropathic pain model

Authors: *M. A. WAGNER, J. E. HOLDEN;
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Abstract: The aim of this study was to examine the effect of PH stimulation on nociception in female and male rats in a model of neuropathic pain. Female and male Sprague-Dawley rats received left sciatic nerve ligation to induce the thermal hyperalgesia of neuropathic pain (n = 4 - 8). Fourteen days later, either carbamoylcholine (carbachol; 500nmol in 0.5 µl normal saline) or normal saline for control was microinjected into the left PH of each rat. Left paw withdrawal latency (PWL) from a thermal stimulus was measured at baseline and every 5 minutes for 45 minutes. Two-way repeated measures ANOVA was conducted for statistical analysis using Sigma Stat. Data were excluded if microinjector placement was located outside of the PH. Left PH stimulation with 500 nmol of carbachol produced significant antinociception in left PWL in female neuropathic rats (10.33 ± 0.99 Vs 5.79 ± 0.92 sec for saline control; $p = 0.005$), but not male neuropathic rats (9.50 ± 1.58 Vs 7.36 ± 1.29 sec for saline control; $p = 0.33$). These preclinical findings are suggestive that the effectiveness of PH stimulation may be dependent on sex.

Disclosures: M.A. Wagner: None. **J.E. Holden:** None.

Poster

613. Central Mechanisms of Pain

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 613.15/HH17

Topic: D.02. Somatosensation: Pain

Title: Deep brain stimulation of the nucleus accumbens alleviates acute inflammatory pain

Authors: *H. HARRIS, J. N. STRAND, A. S. PARCHURE, Y. B. PENG;
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Abstract: The assertion of involvement of the nucleus accumbens in pain processing has been supported by findings of projections to and from the thalamus, somatosensory cortex, and anterior cingulate cortex, which send and receive signals along dopaminergic and glutamatergic pathways (Salgado et al., 2015). One specific affective network through the nucleus accumbens is an indirect pathway known to be associated with negative affect and acute aversive events (Ren et al., 2016). Because of suggested associations between the nucleus accumbens and pain

processing, researchers have begun to examine analgesic effects of treatments such as deep brain stimulation targeted toward the nucleus accumbens and other striatal regions (Mallory et al., 2012). It has previously been shown to be effective for treating Parkinson's symptoms, OCD, depression, and chronic pain (Flora et al., 2010; Nauczyciel et al., 2013; Sturm et al., 2003). Furthermore, research from our laboratory has successfully produced an analgesic effect with deep brain stimulation of the VTA (Li et al., 2016), a structure known to project to the nucleus accumbens. The purpose of this study is to provide evidence of analgesia via stimulation in the nucleus accumbens. The hypothesis of the current study is that deep brain stimulation of the nucleus accumbens will alleviate acute inflammatory pain. To test this hypothesis, electrodes were implanted into the core of the left nucleus accumbens in male Sprague-Dawley rats. After seven days, the rats were tested for noxious heat by thermal paw withdrawal threshold test (TPWT) in order to demonstrate that implantation alone did not affect pain response, and to establish a baseline. Rats were given subcutaneous injections of .05mL of 3% formalin in the right hindpaw. A wireless stimulation module was attached to the implanted electrode. The TPWT was measured again before and after turning on the electrical stimulation at 1 ms pulse width, 100 Hz, and 5 V. Results show paw withdrawal latency significantly increased after stimulation of the nucleus accumbens ($p < .001$), indicating analgesia. However, there was still a significant difference between the latency of the right hindpaw post-stimulation and the left control hindpaw post-stimulation ($p = .003$), indicating that pain was alleviated by deep brain stimulation, but not totally reversed. In conclusion, intracranial stimulation of the nucleus accumbens appears to reduce acute pain response.

Disclosures: H. Harris: None. **J.N. Strand:** None. **A.S. Parchure:** None. **Y.B. Peng:** None.

Poster

614. Molecular Mechanisms of Itch and Pain

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Topic: D.02. Somatosensation: Pain

Support: JSPS KAKENHI 15K15203

Title: Purinergic receptor P2X3 in MrgprA3-positive sensory neurons mediates the itch sensation through a pathway involving GRP receptors

Authors: *M. SHIRATORI-HAYASHI[1], A. HASAGAWA[1], H. TOYONAGA[1], T. ANDOH[3], Y. KURAISHI[3], K. INOUE[2], X. DONG[4], M. TSUDA[1];
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Toyama, Japan; [4]Howard Hughes Med. Institute, Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Itch (or pruritus) is a skin sensation that provokes desire to scratch. The underlying mechanisms for the induction of itch are still unclear. ATP is an energy source and plays an important role in the cell signaling. ATP produces several physiological actions through purinoceptors. P2X3 receptors (P2X3Rs), a member of ATP-gated cation channels as a purinoceptor, are predominantly expressed in small-diameter primary afferent sensory neurons. A growing body of literature has indicated the participation of this channel in nociception. However, the role of P2X3Rs in itch remains unclear. In the present study, we investigated the function of P2X3Rs as itch-related receptors in primary sensory neurons. Intradermal injection of either αβ meATP (a P2X3R agonist) or ATP (an endogenous agonist of P2X3Rs) into the cheek of mice induced scratching, an itch-related response. A317491, a specific antagonist of P2X3Rs, inhibited αβ meATP-evoked scratching. Immunohistochemical analyses using *Mrgpra3*$^{GFP\text{-}Cre}$ transgenic mice revealed that some P2X3Rs were expressed in MrgprA3-positive neurons which are itch specific sensory neurons. Inactivation or deletion of MrgprA3-positive sensory neurons resulted in reduction of P2X3R-mediated scratching. Furthermore, scratching induced by P2X3R agonists was attenuated in mice lacking receptors for gastrin-releasing peptide (GRP), an itch-inducing neuropeptide in the spinal dorsal horn. These findings indicate that activation of P2X3Rs in MrgprA3-positive primary afferent sensory neurons elicits the itch sensation through a pathway involving GRP receptors in dorsal horn neurons.

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Poster

614. Molecular Mechanisms of Itch and Pain

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Topic: D.01. Sensory Disorders

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ARCS Scholarship

Title: Eosinophils increase thymic stromal lymphopoietin receptor expression in sensory nerves: a potential mediator of itch

Authors: *Q. ROTH-CARTER[1], J. J. LEE[4], A. D. FRYER[2], D. B. JACOBY[3];
[1]Cell and Developmental Biol., [2]Physiol. and Pharmacol., [3]Pulmonary and Critical Care Medicin, Oregon Hlth. & Sci. Univ., Portland, OR; [4]Mayo Clin., Scottsdale, AZ

Abstract: Atopic dermatitis is an inflammatory disease of the skin primarily characterized by chronic itch. Our ability to develop effective therapies has been seriously limited by our lack of understanding of the neurobiology of itch in this disease. Itch is mediated by cutaneous sensory nerves, and interactions between sensory nerves and the immune system are key for the development of chronic itch in atopic dermatitis. Using a mouse model of atopic dermatitis we have found that the eosinophil (an immune cell), and specifically the eosinophil granule protein eosinophil peroxidase (EPX), are required for itch in atopic dermatitis. Supporting this finding, eosinophils are elevated and localize to sensory nerves in the skin of patients with atopic dermatitis. However, the mechanism by which eosinophils activate sensory nerves to induce is unknown. A recent finding that cytokine thymic stromal lymphopoietin (TSLP) activates sensory nerves to induce itch led to our hypothesis that eosinophils induce itch in atopic dermatitis by increasing TSLP expression and enhancing TSLP signaling in nerves supplying skin. To address this hypothesis primary cultures of keratinocytes from skin were treated with EPX and its substrates hydrogen peroxide and bromide, and 12 hours later TSLP was measured. EPX and its substrates hydrogen peroxide and bromide significantly increased TSLP gene expression in keratinocytes, and significantly increased TSLP in media. Neither EPX alone, nor hydrogen peroxide and bromide had any effect on TSLP. In separate experiments, co-culture of eosinophils with sensory nerves from dorsal root ganglia significantly increased gene expression for TSLP receptors in sensory nerves. These data show that eosinophils and eosinophil proteins are sufficient to induce expression of TSLP in keratinocytes and TSLP receptors in sensory nerves. These data suggest that eosinophils may mediate itch in atopic dermatitis by increasing TSLP in skin and TSLP receptors on sensory nerves, and that eosinophils and TSLP receptors may be therapeutic targets to treat itch in atopic dermatitis.

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Poster

614. Molecular Mechanisms of Itch and Pain

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Topic: D.02. Somatosensation: Pain

Support: Pfizer grant

NIH grant AR063228

Title: Role for NRTN in spontaneous itch and density of nonpeptidergic intraepidermal fibers in a mouse model of psoriasis

Authors: K. SAKAI[1], K. SANDERS[1], M. YOUSSEF[1], L. JENSEN[2], G. YOSIPOVITCH[1], *T. AKIYAMA[3];
[1]Dermatol., [2]Microbiology and Immunol., [3]Dermatol. & Anat. and Cell Biol., Temple Univ., Philadelphia, PA

Abstract: Chronic itch is the most troublesome symptom of psoriasis, a chronic autoimmune skin disease of scaling and inflammation. The imiquimod-induced psoriasis mouse model has been reported as a useful model to investigate chronic itch in psoriasis. Neurotrophins are thought to be involved in chronic itch through increasing the density of epidermal nerve fibers. We presently investigated if neurotrophins affect the density of intraepidermal fibers and are involved in chronic itch in the imiquimod-induced mouse model of psoriasis. Psoriasis-like skin lesions were produced by daily topical application of imiquimod on the back skin of adult C57BL/6 mouse. Over the time course of imiquimod treatment, the density of epidermal nonpeptidergic nerves significantly increased, while the density of peptidergic nerves significantly decreased. Epidermal nonpeptidergic nerves expressed GDNF family receptor alpha-2, neurturin (NRTN) receptor. NRTN immunoreactivity increased in the skin of imiquimod-treated mice. To block the biological effects of NRTN, a NRTN-neutralizing antibody was intradermally injected into the imiquimod treatment area before each topical application. The NRTN-neutralizing antibody significantly inhibited the increase in nonpeptidergic nerve density as well as spontaneous scratching, a behavior associated with chronic itch. These results indicate that NRTN contributes to an elongation of nonpeptidergic nerves in the skin of imiquimod-treated mice that is likely involved in chronic itch. Therefore, inhibition of NRTN could be a potential treatment for chronic itch in psoriasis.

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Poster

614. Molecular Mechanisms of Itch and Pain

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant 1R01NS083702

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NIH Grant K12-GM-081259

Title: Optogenetic activation of ear innervating MRGPRD$^+$ neurons induces itch behavior

Authors: *P. DONG[1], I. ABDUS-SABOOR[1], W. OLSON[1], L. DING[1], W. YANG[2], T. RAABE[1], M. MA[1], Q. LIU[2], W. LUO[1];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Washington Univ., St. Louis, MO

Abstract: Itch is a unique sensory experience that elicits a desire to scratch. However, pathological itch can arise from a wide variety of neurological disorders and become debilitating. Itch can be classified as either histaminergic or nonhistaminergic based on the involvement of histamine, a pro-inflammatory compound released from immune cells. While most existing research has focused on investigating histaminergic itch, the molecular and circuit mechanisms governing nonhistaminergic itch are still poorly understood. Recent work has identified the MRGPR family of G protein coupled receptors (GPCRs) as the main receptors for detecting nonhistaminergic itch inducing compounds. Many of these GPCRs are expressed by the primary sensory neurons that convey itch, which include small diameter C-fiber dorsal root ganglion (DRG) and trigeminal ganglion (TG) neurons that carry information into the spinal cord and medulla. A subset of these neurons express MRGPRD and are polymodal nociceptors in that they mediate various modalities of both pain and itch at the behavioral level. However, how such a task is accomplished is unclear.
Here, we generated a new mouse line in which an inducible Cre-recombinase is knocked into the *Mrgprd* locus. We were then able to specifically express channelrhodopsin (ChR2) in MRGPRD$^+$ neurons and use blue light to activate their peripheral terminals in the ear skin of awake and behaving mice, showing that these mice preferentially scratched the area of stimulation after light exposure. To our knowledge, our experiments are the first example of optogenetically induced itch, and provide evidence that a subset of neurons are preferably tuned to mediate itch within the MRGPRD$^+$ neuron population. In addition, we demonstrate that optogenetically activated itch lasts for at least an hour after stimulation, suggesting the existence of mechanisms for generating the persistence of itch sensation. Furthermore, compared with the use of direct chemical injections for inducing itch, our optogenetic method is less invasive, temporally precise, and the population of activated neurons is unambiguous. Thus, our work lays a foundation for further study of the mechanisms underlying nonhistaminergic itch.

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Poster

614. Molecular Mechanisms of Itch and Pain

Location: Halls B-H

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Program#/Poster#: 614.05/II5

Topic: D.01. Sensory Disorders

Title: A TRPV1 blocker riboflavin (vitamin B2) for histamine itch relief

Authors: *S. JUNG;
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Abstract: Riboflavin has various therapeutic effects, such as anti-cancer, anti-oxidant, and anti-inflammatory activities. Riboflavin is considered to be important for blockage of transient receptor potential vanilloid 1 (TRPV1), which plays an important role in itch and pain. While riboflavin is known to have therapeutic effects, histamine, a major agitator in various skin diseases, provokes itching by exciting a subset of sensory neurons, predominantly C-fibers. In the recent study, we investigated the effects of riboflavin on itch behaviors in histamine-itching mouse model, and as an attempt to explain its mechanism, we characterized inhibitory effects of riboflavin on TRPV1 in dorsal root ganglion (DRG) neurons. DRG neurons were classified into two types on the basis of their neurochemical and electrophysiological properties such as cell size, and inward current of capsaicin and histamine by using whole-cell patch clamp. Riboflavin reversibly inhibited TRPV1 current by capsaicin and histamine in TRPV1+/Capsaicin+/Histamine+ and TRPV1 transfected HEK293 cells. Effects of riboflavin were also observed in vivo, where itching behavior was reduced in riboflavin-fed mice. However, riboflavin failed to diminish Hargreaves test, which was attributable to the blockade of other TRPV1 pathways. Taken together, these results suggest that riboflavin may serve as an anesthetic agent for other itching conditions. Key word. Riboflavin, TRPV1, Itch, Histamine, Capsaicin, channel blocker

Disclosures: S. Jung: None.

Poster

614. Molecular Mechanisms of Itch and Pain

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Topic: D.02. Somatosensation: Pain

Support: Japan Agency for Medical Research and Development

Title: Thromboxane A_2 increased in atopic dermatitis-like skin lesions induces itch-associated responses through TP receptors on primary afferents in mice

Authors: *T. ANDOH[1], A. YAMAMOTO[1], Y. KURAISHI[2];
[1]Univ. Toyama, Toyama, Japan; [2]Tokyo Med. Dent. Univ., Tokyo, Japan

Abstract: Atopic dermatitis (AD) is a severe and chronic inflammatory skin disease. The itching is its major symptom in AD patients. The scratching induced by the itch sensation aggravates dermatitis and furthermore increases the frequency of itching. Therefore, itch control is very important to improve quality of life and to treat AD. However, the underlying mechanisms of itching in AD patients are still unknown completely. Thromboxane (TX) A_2 is an arachidonic acid metabolite produced by catalysis with COX and TX synthase (TXSyn). Our previous report has shown that an intradermal injection of TXA_2 analogue U-46619 elicits itch-related responses in mice, suggesting that TXA_2 is an itch mediator. In this study, to investigate the mechanisms underlying itch in AD, we examined whether TXA_2 was involved in spontaneous scratching, an itch-related response, in NC mice with AD-like skin lesions (dermatitis mice). The spontaneous scratching in dermatitis mice was inhibited by TP TXA_2 receptor antagonist ONO-3708. The mRNA expression of TXSyn and the concentration of TXB_2, a metabolite of TXA_2, were increased in lesional skin. The immunoreactivity of TXSyn was detected in epidermis, especially stratum spinosum and granular layer. In addition, the primary afferents were elongated into epidermis in dermatitis mice. TP receptor was expressed in primary cultures of dorsal root ganglion (DRG) neurons. U-46619 activated primary cultures DRG neurons. These result suggest that TXA_2 produced in keratinocytes elicits itch-related responses through the activation of TP receptor on primary afferents in dermatitis mice.

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Poster

614. Molecular Mechanisms of Itch and Pain

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Topic: D.01. Sensory Disorders

Support: NIH Grant NS014624

National Natural Science Foundation of China Grant #81271239

Title: MCP-1/CCR2 signaling elicits itch- and pain-like behavior in a murine model of allergic contact dermatitis

Authors: H. JIANG[1,2], C. CHEN[2], S. G. SHIMADA[2], P. ZHANG[2], *C. MA[1], R. H. LAMOTTE[2];
[1]Inst. of Basic Med. Sciences, CAMS&PUMC, Beijing, China; [2]Anesthesiol., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Spontaneous itch and pain are the most common symptoms in various skin diseases, including allergic contact dermatitis (ACD). Monocyte chemoattractant Protein-1 (MCP-1) is a chemokine produced by a variety of non-neuronal cell types during the elicitation phase. The chemokine MCP-1 (CCL2) and its receptor CCR2 are involved in the pathophysiology of ACD; but little is known of the role of MCP-1/CCR2 for the itch and pain accompanying this disorder. C57BL/6 mice previously sensitized to the hapten, squaric acid dibutyl ester, applied to the abdomen were subsequently challenged twice with the hapten delivered to either the cheek or to the hairy skin of the hind paw resulting in contact hypersensitivity (CHS, the murine model of ACD) at that site. By 24 h after the 2nd challenge to the hind paw MCP-1 and CCR2 mRNA, protein, and signaling activity were upregulated in the dorsal root ganglion (DRG). Calcium imaging and whole-cell current-clamp recordings revealed that MCP-1 directly acted on its neuronal receptor, CCR2 to directly activate a subset of small-diameter, nociceptive-like DRG neurons retrogradely labeled from the CHS site. Intradermal injection of MCP-1 into the site of CHS on the cheek evoked site-directed itch- and pain-like behaviors which could be attenuated by prior delivery of an antagonist of CCR2. In contrast, MCP-1 failed to elicit either type of behavior in control mice. Results are consistent with the hypothesis that CHS upregulates MCP-1/CCR2 signaling in a subpopulation of cutaneous small diameter DRG neurons and that MCP-1 can activate these neurons through neuronal CCR2 to elicit itch and pain. Targeting the MCP-1/CCR2 signaling might be beneficial for the treatment of allergic itch and pain in humans.

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Poster

614. Molecular Mechanisms of Itch and Pain

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant R01 DE022358

Title: Investigating the role of Piezo2 in neuropathic pain

Authors: *S. MURTHY, A. FRANCISCO, M. LOUD, A. PATAPOUTIAN;
Neurosci., The Scripps Res. Inst., LA Jolla, CA

Abstract: Neuropathic injury has been known to cause behavioral sensitivity to mechanical stimuli through a combination of central and peripheral sensitization mediated by sensory neurons. However, the molecular mechanism underlying the role of a mechanosensitive ion channel at the periphery remains unknown. We have previously shown that Piezo2, a mechanically activated ion channel, is expressed in a subpopulation of sensory neurons, and is essential for touch sensation and proprioception in mice. In the current study, we investigated the role of Piezo2 in touch-evoked pain after nerve injury. We find that, after subjecting mice to the chronic constriction injury (CCI) neuropathic pain model, mice lacking Piezo2 continue to display mechanical allodynia, a heightened response to low mechanical forces. These results suggest that injury-mediated peripheral sensitization occurs via a pathway independent of Piezo2, or that in the absence of Piezo2 a compensatory mechanism contributes to mechanical hypersensitivity.

Disclosures: S. Murthy: None. **A. Francisco:** None. **M. Loud:** None. **A. Patapoutian:** None.

Poster

614. Molecular Mechanisms of Itch and Pain

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Program#/Poster#: 614.09/II9

Topic: D.02. Somatosensation: Pain

Support: NINDS R01-NS054791

Howard Hughes Medical Institute

NIH T32-NS070201

Title: Mrgb2 contributes to mechanical and thermal allodynia in animal models of inflammatory and neuropathic pain

Authors: *D. GREEN[1], V. TIWARI[2], X. DONG[2];
[1]Neurosci., [2]Johns Hopkins, Baltimore, MD

Abstract: Aim of Investigation: As one of the key effector cells in the inflammatory process, mast cells are an important contributor to pain pathophysiology. The role of mast cells in

mediating postoperative pain is incompletely understood, although studies using mast cell stabilizers demonstrate that this immune cell is involved in contributing to nociception in animal models of postoperative pain. Thus, there is a compelling need for fundamental research aimed at understanding the mechanisms of mast cells in postoperative pain. **Methods:** Towards this objective, our lab has previously published data identifying a novel mast cell specific receptor, Mas-related G-protein-coupled receptor B2 (MRGB2) and its human orthologue, MRGX2, in non-allergenic activation of mast cells. To evaluate the role of MRGB2 in postoperative pain we utilized a hindpaw incision model. After baselines were measured, mice were anesthetized and a 5-mm incision beginning 2 mm from the proximal edge of the right heel was made. Curved forceps elevated the underlying muscle. A mattress suture of 8-0 nylon on a TG175-8 needle were then used to close the incision. Antibiotic ointment (Bacitracin Zinc Ointment) was then applied. As neuropathic pain has also been reported in patients post-surgery we evaluated the role of MRGB2 in nerve injury utilizing a chronic constriction model (CCI). After baselines were measured, mice were anesthetized and the sciatic nerve was exposed at the level of the middle of the thigh by blunt dissection. Three ligatures (silk 6-0) were tied loosely around the nerve with about 1 mm spacing and the incision was closed in layers. **Results:** Male C57bl/6 wild-type (WT) or MRGB2KO (n=6/group) were tested 24 h after incision surgery. Behavioral testing was done utilizing the radiant heat test and Von Frey filaments. At 24 hours post-surgery we saw significant reduction in both mechanical and thermal allodynia in MRGB2 KO mice but not WT. In the CCI model of neuropathic pain, similar results were observed. Behavioral testing was done 14 days after surgery, and we saw significant reduction in both mechanical and thermal allodynia in MRGB2 KO mice but not WT. **Conclusion:** Results indicate that mast cells contribute to postoperative allodynia via MRGB2 and contribute to the thermal and mechanical allodynia seen in a neuropathic pain model. Further study of the mechanisms underlying the role of this receptor in mediating incision induced pain may provide potential strategies for the development of novel analgesics to treat debilitating postoperative and neuropathic pain.

Disclosures: **D. Green:** None. **V. Tiwari:** None. **X. Dong:** None.

Poster

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Support: NSFC grant 81471314

The Important Project of Natural Science in Colleges and Universities in Jiangsu Province 14KJA320002

NSFC Grant 81450064

Title: Slack channel regulate mechanical pain though both dorsal root ganglia and spinal cord mechanism

Authors: ***Z. ZHANG**, F.-F. ZHANG, Y. SONG, R. WANG, J. CHEN, S.-H. GAO, M.-H. SUN, M.-J. TAN, Q.-Y. TANG;
Xuzhou Med. Col., Xuzhou, China

Abstract: Slack (Slo2.2, KCNT1) channels are sodium activated potassium channels that are widely expressed in nervous system, such as dorsal root ganglia, spinal cord and brain. The role of Slack channels in pain sensing is still in debating (Lu R, et al 2015;Huang F, et al 2013). In this abstract, we reported that Slack channels regulate mechanical pain sensing but do not alter thermal or cold pain sensing by examining Slack channel knockout mice. In addition, we found that Slack channels are richly expressed in IB4 positive Drg neurons but are relative lower expressed in CGRP and NF200 positive Drg neurons. Electrophysiological data showed that mediate and small Drg neurons of Slack KO mice have increased firing rate and higher resting potentials than mediate and small Drg neurons of WT mice. But the large Drg neurons of Slack KO mice did not show different excitability with large Drg neurons of WT mice. However, the Slack channels also are richly expressed in Spinal cord, especially in dorsal horn. Immunostaining, in situ hybridization and electrophysiological study showed that spinal cord mechanism is also important for Slack channel's role in pain sensing.

Disclosures: **Z. Zhang:** None. **F. Zhang:** None. **Y. Song:** None. **R. Wang:** None. **J. Chen:** None. **S. Gao:** None. **M. Sun:** None. **M. Tan:** None. **Q. Tang:** None.

Poster

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Topic: D.02. Somatosensation: Pain

Support: BSMS Research Grant

Title: The contribution of transient receptor potential channels to axonal mechanical sensitivity and radiating pain

Authors: *G. GOODWIN, A. DILLEY;
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Abstract: Work from our laboratory has shown that inflammation (neuritis) can cause intact nociceptive axons to become mechanically sensitive (1). Such axonal mechanical sensitivity (AMS) may underlie movement-evoked radiating pain. Axonal transport is disrupted at the neuritis site (2), and we have hypothesised that transported mechanically sensitive ion channels may be accumulating at this site, leading to AMS. In this study, we have examined the physiological properties of mechanically sensitive axons and have begun to identify a potential role for TRP channels. In anaesthetised adult male Sprague Dawley rats, a strip of absorbable gelatin sponge saturated in either 0.1mM vinblastine (n=5) or complete Freund's adjuvant (n=3) was wrapped around the sciatic nerve as previously described (2). Vinblastine disrupts axonal transport in the absence of inflammation or degeneration and causes intact C-fibre axons to develop AMS (2). Three to 5 days post-surgery, the sciatic nerve was extracted and maintained at 32 °C in a recording bath that was perfused with oxygenated synthetic interstitial fluid. Single unit extracellular recordings from teased C-fiber axons were performed. The nerve was mechanically stimulated using a silicone tipped probe. Once a mechanically sensitive 'hotspot' had been located, a feedback-controlled mechanical stimulator (Aurora Scientific) was used to apply increasing forces to the nerve (20-250mN; duration of each stimulus: 2 sec). A ring was positioned over the nerve at each 'hotspot' to allow 10-50μM ruthenium red, a TRP channel blocker, to be applied. Force-discharge relationships were reassessed following the application of these agents. TRPV1 channel expression at the treatment site was also examined using immunohistochemistry. AMS 'hot spots' were identified at the treatment site in vinblastine-treated and neuritis nerves. There was a positive relationship between force applied and firing rate (vinblastine, $r^2 = 0.66$, n=10; neuritis $r^2 = 0.35$, n=7). The mean thresholds for AMS were 79mN (SEM +/- 16) in vinblastine-treated and 47mN (SEM +/- 6) in neuritis nerves. 50uM ruthenium red increased the threshold of mechanical sensitivity by 38.5% (SEM +/- 10) and 31% (SEM +/- 37) respectively. Preliminary immunohistochemical data revealed TRPV1 labelling at the treatment site. In summary, our data has revealed a role for TRP channels in the development of AMS. Therefore, drugs that target TRP channels may be effective in the treatment of radiating pain.
1. Dilley et al 2005, Pain 117: 462-72 2. Dilley et al 2013, J Pain 14:1437-49

Disclosures: G. Goodwin: None. **A. Dilley:** None.

Poster

614. Molecular Mechanisms of Itch and Pain

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Topic: D.02. Somatosensation: Pain

Title: Role of voltage-gated sodium channels and calcium channel subunit alpha-2/delta-1 in the lumbar dorsal root ganglions of persistent peripheral neuropathic pain model mice.

Authors: *H. KABURAGI, A. SUMI, T. HIRAI, Y. WAKABAYASHI, T. YOKOTA, A. OKAWA, M. ENOMOTO;
Tokyo Med. and Dent. Univ., Tokyo, Japan

Abstract: Voltage-gated ion channels are implicated in the development and maintenance of chronic pain. However, their exact roles in chronic pain, particularly in neuropathic pain, have yet to be elaborated as their roles could vary depending on the nature of the pathology and over time. Thus, formulating an ion channel-based treatment strategy will depend on understand the role of ion channels across a number of painful neuropathies and their chronological expression. The current study quantified changes over time of voltage-gated sodium channels (Nav) and the Ca2+ channel α2δ-1 subunit in lumbar dorsal root ganglia (DRG) in mice with either a spared nerve injury (SNI) or a partial sciatic nerve ligation (PSNL). Following nerve injury surgery, hind paw responsiveness to mechanical, cold and heat stimulation were assessed weekly. Following behavioral testing, total RNA was extracted from L3-L5 DRG and expression of Nav. 1.7, 1.8, 1.9 and calcium channel α2δ-1 subunit was quantified with real-time PCR. Following SNI, mice exhibited significantly increased sensitivity to tactile and cold stimuli (hyperalgesia) within 3 days of surgery, which lasted 6 weeks after surgery. PSNL mice gradually increased cold hyperalgesia after surgery. Heat hyperalgesia was observed in both models for the duration of the observational period. Expression of Nav1.7 mRNA in either model did not change over the course of the observational period. Expression of Nav1.8 and Nav1.9 mRNA in the PSNL was decreased three days after surgery. Compared to control (non-injured) mice, Nav1.8 and Nav1.9 in both models were decreased 25% and 27% respectively three weeks after injury. Six weeks after injury, expression of Nav1.8 and Nav1.9 in both models did not significantly differ from that of control mice. Compared to control mice, expression of α2δ-1 mRNA in both models was immediately increased three days and about 3-fold beginning 1 week after surgery. Six weeks after injury, SNI mice showed higher expression of α2δ-1 compared to PSNL mice. The current findings suggest that Nav1.8 and α2δ-1 contribute to the transition from an acute to chronic pain state since their expression in both neuropathic pain models changed over time. However, 6 weeks after surgery, α2δ-1 expression was still significantly altered, whereas Nav expression returned to control levels. The findings suggest that targeting α2δ-1 expression, rather than Nav, during the chronic phase could be crucial in alleviating persistent neuropathic pain.

Disclosures: H. Kaburagi: None. **A. Sumi:** None. **T. Hirai:** None. **Y. Wakabayashi:** None. **T. Yokota:** None. **A. Okawa:** None. **M. Enomoto:** None.

Poster

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant 5T32NS007484

Title: Functional and transcriptional changes in somatosensory neurons after peripheral nerve injury.

Authors: *I. TOCHITSKY[1,3], S. LEE[3], I. CHIU[2], C. WOOLF[3];
[1]Harvard Med. Sch., Brookline, MA; [2]Microbiology and Immunol., Harvard Med. Sch., Boston, MA; [3]Neurobio., Boston Children's Hosp., Boston, MA

Abstract: Neuropathic pain is a common disorder typically caused by disease of or damage to the peripheral nervous system. Spontaneous pain is a major feature of neuropathic pain disorders. This ongoing pain is thought to be generated by the ectopic activity of peripheral somatosensory neurons which is caused not by any external stimulus but rather the intrinsic hyperexcitability of injured neurons. Unfortunately, our current understanding of this ectopic activity is incomplete. In particular, it is not known which subtypes of peripheral sensory neurons become spontaneously active after nerve injury or what changes in gene expression cause their hyperexcitability. Here, we present functional and transcriptional data exploring the changes that take place in injured somatosensory neurons and drive their hyperexcitability. We used the spared nerve injury in mice as an animal model of neuropathic pain. Spared nerve injury leads to pain behaviors such as heat and mechanical hyperalgesia, and mechanical allodynia, which are also present in patients with neuropathic pain. One week after injury, we dissected and cultured the ipsilateral injured and contralateral uninjured lumbar L3 and L4 dorsal root ganglia (DRG) neurons *in vitro* and then performed a range of functional and transcriptional analyses on these cultured neurons. We observed a higher incidence of spontaneous activity in the ipsilateral DRG neurons as compared to the contralateral DRG neurons using both patch clamp electrophysiology and calcium imaging methods. Additionally, the injured DRGs were less excitable to a variety of electrical stimuli. We also observed a lower current density of voltage gated potassium currents in the ipsilateral DRG neurons, which would be consistent with their hyperexcitability. We confirmed the deficit in potassium channel function using the thallium assay, which involves imaging potassium flux into DRG neurons. Finally, we performed a transcriptional analysis on the injured vs uninjured DRG neurons and found a number of differences in ion channel expression that are associated with injury-induced hyperexcitability. We believe that our combined functional and transcriptional analysis may help identify new drug targets for the treatment of neuropathic pain and may lead to the development of improved therapeutics.

Disclosures: **I. Tochitsky:** None. **S. Lee:** None. **I. Chiu:** None. **C. Woolf:** None.

Poster

614. Molecular Mechanisms of Itch and Pain

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant R01 NS87988

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Anesthesiology Departmental Startup Funds (TV)

Title: Optogenetic control of Aβ-fibers in acute and chronic pain conditions

Authors: ***A. CHAMESSIAN**[1], M. W. WANG[2], Y.-H. KIM[1], T. VAN DE VEN[1], R.-R. JI[1]; [1]Anesthesiol., [2]Duke Univ., Durham, NC

Abstract: The ability to gain genetic access to light touch-sensing Aβ primary sensory neurons would be broadly enabling to the study of normal and pathological sensory processing. To that end, we have characterized a new knockin-in Cre-driver line that expresses under the control of the Vesicular Glutamate Transporter 1 (VGLUT1) gene locus. To examine whether VGLUT1-Cre shows faithful expression in VGLUT1-expressing (dorsal root ganglia) DRG neurons, we crossed VGLUT1-Cre with a floxed Channelrhodopsin-EYFP (ChR2) reporter line (Ai32). The resulting cross, VGLUT1-ChR2, both marks VGLUT1-positive neurons and also allows for optogenetic control of this neuronal population. Extensive characterization of the VGLUT1-ChR2 line by immunofluorescence microscopy demonstrated that VGLUT1-ChR2 is expressed in medium- and large-sized DRG neurons and colocalizes with Parvalbumin, Ret, Piezo2, and Neurofilament 200 (NF200). Moreover, the ChR2-EYFP neurons showed virtually no colocalization with the nociceptive markers IB4, Peripherin, and CGRP, demonstrating that ChR2-EYFP expression is not present in nociceptors. In the spinal cord, VGLUT1-ChR2 fibers were observed specifically in the deep dorsal horn (lamina III-V), dorsal column, and to a lesser extent, the ventral horn, consistent with the features of myelinated A-fibers. In the skin, ChR2-EYFP is present in Meisnner Corpuscles, Hair Follicles and Merkel Cell-Neurite complexes, indicating that the VGLUT1-ChR2 population encompasses both rapidly adapting and slowly adapting subtypes of Aβ fiber. Optogenetic photostimulation of the plantar hindpaw in awake, behaving animals showed clear innocuous responses to photostimulation, namely paw lifting and ambulation, but never any nocifensive responses such as licking, biting, flinching or jumping.

Taken together, these results strongly suggest that the VGLUT1-ChR2 line is a specific tool for the manipulation of low-threshold A-fibers. With this tool in hand, we are now using the VGLUT1-ChR2 mouse to interrogate the specific contributions of Aβ fibers to pain in models of inflammatory and neuropathic conditions.

Disclosures: A. Chamessian: None. **M.W. Wang:** None. **Y. Kim:** None. **T. Van de Ven:** None. **R. Ji:** None.

Poster

614. Molecular Mechanisms of Itch and Pain

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Topic: D.02. Somatosensation: Pain

Support: Guadalupe Garcia is a Conacyt fellow

Title: Expression of bestrophin-1 in dorsal root ganglion neurons after nerve injury in the rat.

Authors: *J. MURBARTIAN, G. GARCÍA;
Cinvestav, Sede Sur, Mexico, DF, Mexico

Abstract: The calcium-activated chloride channels (CaCC) are involved in several physiological processes including neuronal regeneration and sensory transduction. Four genes encoding for bestrophins (Best) have been reported, the first channels to settle within CaCC family. Although Best-1 has been involved in neuropathic pain, the expression profile in individual dorsal root ganglia (DRGs) of this channel is lacking. The purpose of this study was to determine the expression of Best-1 in individual L4, L5 and L6 DRGs in rats subjected to the spinal nerve ligation (SNL). Best-1 was expressed in L4, L5 and L6 DRGs of naïve and sham-operated rats. SNL increased Best-1 protein expression in L5 and L6, but not in L4, DRGs. Of note, SNL enhanced Best-1 protein expression in L5 DRG at 1 day after nerve injury, while it increased Best-1 protein expression in L6 DRG at 14 and 21 days after injury. Likewise, SNL rose protein expression of the nerve injury marker activating transcription factor 3 (ATF3) in L5 and L6, but not L4, DRGs. Since Best-1 has been involved in nerve regeneration and pain, our results suggest that Best-1 channels could contribute to axonal regeneration and nociceptive processing after nerve injury.

Disclosures: J. Murbartian: None. **G. García:** None.

Poster

614. Molecular Mechanisms of Itch and Pain

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Topic: D.02. Somatosensation: Pain

Title: Participation of TRPC in pronociceptive effect induced by hydrogen sulfide in hyperglycemic rats

Authors: *J. E. ROA-CORIA[1], F. J. FLORES-MURRIETA[1], V. GRANADOS-SOTO[2], H. I. ROCHA-GONZALEZ[1];
[1]Superior Sch. of Med. IPN, México, D.F., Mexico; [2]CINVESTAV-SUR, Ciudad de México, Mexico

Abstract: Hydrogen sulfide (H_2S) is a gasotransmitter that produces a pronociceptive effect in many nociceptive models, but little is known about the molecular targets involved in such effect. The aim of this work was to establish the participation of TRPC receptor in the nociception induced by H_2S in peripheral neuropathy associated with diabetes. Hyperglycemia was induced by streptozotocin (60 mg/kg, ip) in Wistar rats, which had values of glucose >350 mg/dL within 2 weeks. Nociception was evaluated by injection of 0.5 % formalin into the right hind paw of the rat. Local peripheral ipsilateral, but not contralateral, injection of H_2S (100 µg/paw) increased nociceptive behavior. Moreover, pronociceptive effect of H_2S was prevented by local administration of SKF96365 (TRPC receptor antagonist; 30, 100 µg/pata) and GsMTx4 (TRPC1 and TRPC6 receptor selective antagonist; 1, 5.6 µg/paw). These data suggest that the local pronociceptive effect induced by H_2S in rats with peripheral neuropathy could be mediated by activation of TRPC receptors, so the use of blockers of these channels may be a useful strategy to treat painful peripheral neuropathy associated with diabetes.

Disclosures: J.E. Roa-Coria: None. **F.J. Flores-Murrieta:** None. **V. Granados-Soto:** None. **H.I. Rocha-Gonzalez:** None.

Poster

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant DA-K01-024751

IRP, NIDA, NIH/DHHS

Title: Sigma-1 receptor affects neuropathic pain by modulating neuronal excitability in the primary sensory neurons

Authors: *H.-E. WU[1], B. PAN[2], H.-W. YU[2], Q. H. HOGAN[2], T.-P. SU[1];
[1]IRP/NIDA/NIH, Baltimore, MD; [2]Anesthesiol., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: We have recently demonstrated that the sigma-1 receptor (σ1R) is present in dorsal root ganglions (DRG) and that the activation of the σ1R in DRG diminishes I_{Ca} and intracellular Ca^{2+} store which are known features of painful injury in primary sensory neurons. The present study explored the role of σ1R in the DRG as it relates to neuropathic pain at the pharmacological, electrophysiological, and molecular biological level. (A) The σ1R agonist (+)-pentazocine or antagonist BD1047 (4μg/2μl) was injected directly into the 4th and 5th lumbar (L4, L5) DRGs. (+)-Pentazocine produced a hypersensitivity to threshold mechanical stimulation and also a trend toward hypersensitivity to noxious mechanical, brush or cold stimulation. In spinal nerve ligation (L5 & L6) rats, BD1047 diminished neuropathic hypersensitivity. (B) To explore the σ1R agonistic effect on the neuronal excitability, (+)-pentazocine was tested in a system using intact DRG recording and dissociated sensory neurons. (+)-Pentazocine (10 μM) decreased both the AHP amplitude and duration which allowed for more APs following high frequency depolarizing stimuli. Those findings indicate that the σ1R controls the DRG excitability in that the σ1R agonist increases the neuronal excitability in DRG. (C) (+)Pentazocine (100 μM) caused an inhibition of activity-induced (50 mM K^+, 3s) Ca^{2+} transients in DRG neurons; the reduction of the Ca^{2+} transient is known to relate to neuropathic pain. Interestingly, this inhibition caused by (+)pentazocine was not seen in DRG neurons transfected by AAV6-σ1R shRNA. When taken together, the above results suggest that the σ1R participates in the DRG-generated neuropathic pain by regulating the excitability of DRG. (Supported in part by the IRP, NIDA, NIH/DHHS)

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Poster

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Topic: D.02. Somatosensation: Pain

Title: Deciphering the molecular complexity of DRG neurons using single cell molecular profiling technology

Authors: *N. ALESSANDRI-HABER, R. BREESE, Y. BAI, W. FURY, M. SIVULA, Y. WEI, M. NI, C. ADLER, C. LIN, A. MURPHY, L. MACDONALD;
Regeneron Pharmaceuticals Inc, Tarrytown, NY

Abstract: Chronic painful conditions affect millions of people worldwide. Patients suffering from chronic pain often experience hypersensitivity to mechanical, thermal and/or chemical stimulation in the form of hyperalgesia and/or allodynia. This hypersensitivity is mediated, at least in part, by sensitization of signaling processes in different subpopulations of primary sensory neurons known as nociceptors. The cell bodies of these neurons are clustered in nodules on dorsal roots of the spine known as dorsal root ganglia (DRG). The axons of these neurons split into two branches; one branch innervates the peripheral tissues (skin, muscles, joints and organs) and the other connects to the spinal cord to carry signals to the appropriate integration center in the brain. Nociceptors fall into different subpopulations based on their conduction properties, molecular profile and whether they respond to a single type of physical stimulus or integrate and generate a response to different types of stimuli. Although the development of chronic pain has been associated with increased excitability of nociceptors, their molecular complexity has made it difficult to fully elucidate the critical mechanisms underlying persistent pain. To get a better understanding of the molecular complexity within the different subpopulations of DRG neurons, we utilized the Fluidigm C1™ single-cell RNA sequencing system. We show here that we can reproducibly discriminate different subpopulations of DRG neurons and correlate them to known functional subclasses of sensory neurons. These distinct subpopulations of neurons are still reproducibly detected following peripheral injections of the inflammagen carrageenan or i.p. injection of the chemotherapy drug, Paclitaxel. Moreover, we show that distinct subpopulation of neurons display different gene signatures in an animal model of Paclitaxel-induced neuropathy. Our results suggest that single cell molecular profiling technology could help unravel the molecular mechanisms occurring in distinct subpopulations of DRG in the setting of neuropathic pain.

Disclosures: **N. Alessandri-Haber:** A. Employment/Salary (full or part-time): Regeneron Pharnaceuticals, INc. **R. Breese:** A. Employment/Salary (full or part-time): Regeneron Pharmaceuticals Inc. **Y. Bai:** A. Employment/Salary (full or part-time): Regeneron Pharmaceuticals, Inc. **W. Fury:** A. Employment/Salary (full or part-time): Regeneron Pharmaceuticals, Inc. **M. Sivula:** A. Employment/Salary (full or part-time): Regeneron Pharmaceuticals Inc. **Y. Wei:** A. Employment/Salary (full or part-time): Regeneron Pharmaceuticals, Inc. **M. Ni:** A. Employment/Salary (full or part-time): Regeneron Pharmaceuticals, Inc. **C. Adler:** A. Employment/Salary (full or part-time): Regeneron Pharmaceuticals Inc. **C. Lin:** A. Employment/Salary (full or part-time): Regeneron Pharmaceuticals Inc. **A. Murphy:** A. Employment/Salary (full or part-time): Regeneron Pharmaceuticals Inc. **L. Macdonald:** A. Employment/Salary (full or part-time): Regeneron Pharmaceuticals Inc.

Poster

614. Molecular Mechanisms of Itch and Pain

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Program#/Poster#: 614.19/JJ2

Topic: D.02. Somatosensation: Pain

Title: Development of a high capacity assay for systematic silencing of pain targets in DRG sensory neurons using RNAi

Authors: *C. LINDWALL-BLOM, C. NODIN, Å. JÄGERVALL, J. PIHL, J. SVENSSON DALÉN, S. LARDELL, K. ANNA, M. KARLSSON, P. KARILA;
Cellectricon AB, Mölndal, Sweden

Abstract: The introduction of nucleic acids in to living cells has become a groundbreaking toolbox for exploring gene function. In example RNA interference (RNAi), a form of post-transcriptional gene silencing provides a powerful tool to explore pathway, gene and protein function in cell cultures. However, the key for successful RNAi experiments is efficient delivery of the siRNA into the cell type of interest, and in biologically relevant cells models, which are typically primary and/or non-dividing, transfection can be highly challenging.
Electroporation is an efficient method for nucleic acid delivery that may be applied for a variety of cell types. Transfection of primary cells is however generally challenging. Using Cellaxess® Elektra platform, we can efficiently transfect adherent primary cells in vitro in high density microplates. In this particular case, our aim was to develop a plate-based assay in 384-well format for screening of RNAi knockdown in cultured rat dorsal root ganglia (DRG) neurons, targeting genes in the pain pathway.
For this purpose, we used the multiple capabilities of the Cellaxess® Elektra platform. siRNA was delivered to cultured rat dorsal root ganglia (DRG) neurons followed by electric field stimulation (EFS) and detection/imaging of the excitability response using a calcium indicator. Following this, cultures were immunolabeled for nociceptive neuron identity (RIIb) and read on a high content imaging platform. These images were then aligned with the EFS data which enabled analysis of neuronal excitability of the nociceptive neurons only. siRNAs were selected to target pain relevant genes, including the voltage gated sodium channel NaV1.7, the nerve growth factor (NGF) receptor tyrosine receptor kinase A (TrkA), and growth-associated protein 43 (GAP43). A non-targeting control and technical control siRNA was used for reference purposes.
Taken together, this study demonstrates the multiple applicability of the Cellaxess® Elektra® platform for efficient identification of novel pain targets in a physiologically relevant context.

Disclosures: **C. Lindwall-Blom:** A. Employment/Salary (full or part-time): Cellectricon AB. **C. Nodin:** A. Employment/Salary (full or part-time): Cellectricon AB. **Å. Jägervall:** A.

Employment/Salary (full or part-time): Cellectricon AB. **J. Pihl:** A. Employment/Salary (full or part-time): Cellectricon AB. **J. Svensson Dalén:** A. Employment/Salary (full or part-time): Cellectricon AB. **S. Lardell:** A. Employment/Salary (full or part-time): Cellectricon AB. **K. Anna:** A. Employment/Salary (full or part-time): Cellectricon AB. **M. Karlsson:** A. Employment/Salary (full or part-time): Cellectricon AB. **P. Karila:** A. Employment/Salary (full or part-time): Cellectricon AB.

Poster

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Topic: D.02. Somatosensation: Pain

Support: Minnesota Medical Foundation

Title: Deletion of novel peptide TMEM35 alters pain signaling in mice

Authors: *P. V. TRAN[1], B. C. KENNEDY[2], W. V. HOHENBERG[3], J. C. GEWIRTZ[3]; [1]Dept. of Pediatrics, [2]Program in Neurosci., [3]Dept. of Psychology, Univ. of Minnesota, Minneapolis, MN

Abstract: Chronic pain affects over 100 million adults in the USA, with considerable economic costs in healthcare and loss of productivity. Notable progress has been made in elucidating neural mechanisms underlying pain perception, leading to important insights into the maladaptive changes that produce persistent or chronic pain. Nevertheless, there is still potential benefit for medical discovery to generating additional molecular targets for alternative treatment strategies. We investigated a novel and evolutionarily conserved polypeptide (TMEM35) that is strongly expressed in developing and adult pain networks (i.e., dorsal root ganglia (DRG) and spinal cord). Given the persistently reduced TMEM35 expression in the spinal cord following spinal cord injury, the potential role for TMEM35 in neuropathic pain was probed by assessing whether TMEM35 protein levels in the spinal cord are altered by spared nerve injury, a model of chronic pain. Additionally, we generated a *tmem35* knockout (KO) mouse and measured pain sensitivity utilizing various stimuli, including Hargreaves, von Frey, tail flick, and temperature preference. Relative to wild type (WT) mice, KO mice exhibited heightened pain sensitivity across all behavioral measures, indicating a role for TMEM35 in pain signaling. Finally, we quantified expression of peptidergic and non-peptidergic nociceptors in the DRG and projections to the spinal cord, and found that deletion of TMEM35 alters characteristics of the peripheral pain networks. Collectively, our findings suggest that TMEM35 may function to protect against

hyperalgesia in normal animals. Further investigation is needed to determine the molecular role of TMEM35 in pain networks.

Disclosures: **P.V. Tran:** None. **B.C. Kennedy:** None. **W.V. Hohenberg:** None. **J.C. Gewirtz:** None.

Poster

614. Molecular Mechanisms of Itch and Pain

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Topic: D.02. Somatosensation: Pain

Support: NS023725

T32 NS073548

Title: Functional characterization of two overlapping populations of cutaneous afferent fibers by optogenetic activation

Authors: ***M. WRIGHT**, K.-H. LEE, P. C. ADELMAN, H. KOERBER;
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Abstract: Sensory neurons in mammalian dorsal root ganglia (DRGs) can be organized and characterized based on many criteria, including their end organ innervation patterns, physiological response characteristics, and gene expression profiles. Here, we characterized the response properties following optogenetic activation of two partially overlapping subpopulations of small diameter sensory neurons. *Trpv1*^{Cre} and *Mrgd*^{Cre} mice were each crossed to the channelrhodopsin-YFP reporter (*Ai32*), enabling their histological examination and selective activation by exposure to blue light. The *Trpv1*^{Cre} line labels all *Trpv1*-lineal afferents, encompassing both peptidergic and nonpeptidergic small diameter fiber subpopulations, including many Mrgd-expressing neurons. *Mrgd*^{Cre} mice similarly label all *Mrgd*-lineal afferents, including those that express Mrga3, though labeling is limited to putative nociceptors. Using an *ex vivo* DRG-saphenous nerve-skin preparation, we evaluated the impact of blue light on ChR2-expressing cells and determined both that light activation elicits indistinguishable activity in each Cre line and that this activation is comparable to responses seen following mechanical and thermal stimulation. Surprisingly, our initial behavioral assays show that *Trpv1*^{Cre}*;Ai32* mice had a strong withdrawal response to blue light, while *Mrgd*^{Cre}*;Ai32* mice had a much more attenuated response. This led us to investigate how aversive the activation of these two afferent subpopulations is during normal animal behavior. Mice were tested in a novel place-preference

behavioral assay in which a testing chamber was floor-lit with blue light on one side and matched-intensity yellow control lights on the other. Lights were either left on (sustained) or flashed at 2Hz for 15 minutes. On average, *Trpv1^{Cre};Ai32* mice spent < 5% of the entire testing period on the blue lit side of the testing chamber during both light exposure paradigms, indicating a strong aversion to *Trpv1^{Cre}* afferent activation (n=4 mice). In contrast, *Mrgd^{Cre};Ai32* mice showed neither a preference for or aversion to either side of the testing chamber, spending an equal amount of time on both sides (n=10 mice), suggesting that activation of *Mrgd^{Cre}* afferents may not be aversive. Interestingly, preliminary data suggests that spinal nerve ligation (SNL) injury causes activation of *Mrgd^{Cre};Ai32* afferents to become aversive, with *Mrgd^{Cre};Ai32* mice spending significantly less time on the blue lit side of the chamber post-injury (n=6 mice). These results suggest that *Mrgd^{Cre}* afferents may not normally function as true nociceptors, though this may change in the context of nerve injury.

Disclosures: **M. Wright:** None. **K. Lee:** None. **P.C. Adelman:** None. **H. Koerber:** None.

Poster

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Topic: D.02. Somatosensation: Pain

Title: Long-term effects of chronic inflammation on sodium channel gene expression in dorsal root ganglion neurons

Authors: *M. E. O'LEARY, A. BOTTARO, I. KUZIN, C. HO, B. FISCHER;
Rowan Univ. Cooper Med. Sch., Camden, NJ

Abstract: The goal of these studies was to investigate the effects of chronic inflammation on the expression of voltage-gated sodium channels in dorsal root ganglion (DRG) neurons. We employed a transgenic mouse model (TNFtg) that over expresses tumor necrosis factor-alpha (TNFα) resulting in chronic systemic inflammation. After 20 weeks the mechanical sensitivity of the hindpaws of TNFtg animals determined using von Frey filaments increased 3-fold by comparison to control littermates. This is consistent with ambulatory behavior measured in open arena testing which was reduced 3.8-fold in the TNFtg animals. Hargraves testing revealed that the TNFtg mice displayed a significant 31% decrease in hindpaw withdrawal time in response to noxious thermal stimulation. The observed mechanical allodynia and thermal hyperalgesia are hallmarks of plantar nociceptor sensitization. The underlying mechanism was further investigated using single-cell RT-PCR to quantitatively compare the expression of sodium channel transcripts in small-diameter (<25 μm) nociceptors isolated from acutely-dissociated

DRGs of control and TNFtg mice. While the transcripts of many of sodium channels were not altered (Nav1.1, Nav1.6, Nav1.7), a broadly expressed neuronal sodium channel (Nav1.2) and two nociceptor-specific sodium channels (Nav1.8, Nav1.9) were significantly upregulated in the nociceptors of TNFtg animals. Nav1.8 is a high threshold, tetrodotoxin-resistant (TTX-R) sodium channel that drives the rapid upstroke of the nociceptor action potential. Nav1.9 is a low threshold TTX-R sodium channel characterized by slow gating kinetics and substantial overlap of the activation and inactivation relationships that produce persistent inward sodium current at voltages near the resting membrane potential. The combination of a 2-fold increase Nav1.8 and 4-fold increase in Nav1.9 in nociceptors of TNFtg mice is predicted to lower the threshold and potentiate the repetitive firing of action potentials. These data indicate that an increase sodium channel gene expression produced by over-expression of TNFα contributes to the development of chronic inflammatory pain.

Disclosures: **M.E. O'Leary:** None. **A. Bottaro:** None. **I. Kuzin:** None. **C. Ho:** None. **B. Fischer:** None.

Poster

614. Molecular Mechanisms of Itch and Pain

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Topic: D.02. Somatosensation: Pain

Support: National Institute of Neurological Disorders and Stroke Grants NS087542 and NS078530

Title: Inflammatory and neuropathic cold allodynia are selectively mediated by the neurotrophic factor receptor GFRα3

Authors: ***S. ONGUN**[1], E. K. LIPPOLDT[2], G. K. KUSAKA[2], D. D. MCKEMY[2];
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Abstract: Tissue injury prompts the release of a number of proalgesic molecules that induce acute and chronic pain by sensitizing pain-sensing neurons (nociceptors) to heat and mechanical stimuli. In contrast, many proalgesics have no effect on cold sensitivity or can inhibit cold-sensitive neurons and diminish cooling-mediated pain relief (analgesia). Nonetheless, cold pain (allodynia) is prevalent in many inflammatory and neuropathic pain settings, with little known of the mechanisms promoting pain vs. those dampening analgesia. Here, we show that cold allodynia induced by inflammation, nerve injury, and chemotherapeutics is abolished in mice lacking the neurotrophic factor receptor glial cell line-derived neurotrophic factor family of

receptors-α3 (GFRα3). Furthermore, established cold allodynia is blocked in animals treated with neutralizing antibodies against the GFRα3 ligand, artemin. In contrast, heat and mechanical pain are unchanged, and results show that, in striking contrast to the redundant mechanisms sensitizing other modalities after an insult, cold allodynia is mediated exclusively by a single molecular pathway, suggesting that artemin-GFRα3 signaling can be targeted to selectively treat cold pain.

Disclosures: S. Ongun: None. **E.K. Lippoldt:** None. **G.K. Kusaka:** None. **D.D. McKemy:** None.

Poster

614. Molecular Mechanisms of Itch and Pain

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Support: NIH R1 NS065926

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NIH F32 NS096963

Title: Protease activated receptor type 2 (PAR2) mediates nociception in a mouse behavioral model of migraine

Authors: *S. N. HASSLER[1], F. B. AHMED[1], J. VAGNER[2], S. BOITANO[3], T. J. PRICE[1], G. O. DUSSOR[1];
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Abstract: Migraine pain adversely affects millions of people each year in the U.S. and is a significant public health concern. The etiology that causes migraine pain is not well understood but is generally thought to occur as a result of sensitization of afferents that innervate the cranial meninges. Protease activated receptors (PARs) are expressed in trigeminal neurons and dural fibroblasts and have been implicated in other types of pain disorders. PAR2 is endogenously activated by trypsin and tryptase, the latter of which is released by mast cells. Since mast cell degranulation has been proposed to contribute to migraine pain, PAR2 may be a link between immune cell activation and neuronal activity. The current project investigated the role of meningeal PAR2 in migraine using a novel mouse behavioral model and calcium imaging of

trigeminal ganglia cultures. We found that 2-aminothiazol-4-yl-LIGRL-NH_2 (2AT), a PAR2 peptidomimetic agonist increases tactile sensitivity and grimacing, and C391, a PAR2 peptidomimetic antagonist is able to attenuate 2AT mediated nociceptive behaviors when applied onto mouse dura. Further, 2AT activates calcium signaling in primary trigeminal and dural cell cultures (fibroblasts), with C391 attenuating the calcium signaling response to 2AT. When given intravenously, C391 is able to attenuate nociceptive behaviors produced after dural application of compound 48/80, a mast cell degranulator, demonstrating efficacy against an endogenous mechanism thought to contribute to migraine pain. Together, these data show that trigeminal neuron PAR2 signaling contributes to nociceptive behaviors observed in a mouse migraine behavioral model and also show a possible contribution of PAR2 on dural fibroblasts to this process. The study suggests that PAR2-dependent activity in the meninges involves the interaction between neuronal and non-neuronal cells and that these PAR2 effects are a potential therapeutic target for treating migraine pain.
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Poster

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Topic: D.02. Somatosensation: Pain

Support: NS094664

DA033390

Title: DNMT1-mediated epigenetic repression of Kcna2 channel in primary sensory neurons contributes to neuropathic pain genesis

Authors: ***L. SUN**[1], X. GU[2], S. WU[2], L. LIANG[2], B. LUTZ[2], A. BEKKER[2], Y. TAO[2]; [1]Anesthesiol. Department, Rutgers Univ., Newark, NJ; [2]Anestheisology Department, Rutgers Univ., Newark, NJ

Abstract: Peripheral nerve injury-induced downregulation of the voltage-gated potassium channel Kcna2 in dorsal root ganglion (DRG) neurons contributes to neuropathic pain genesis. However, the mechanism of how this occurs is still elusive. DNA methylation that gates gene expression is caused by DNA methyltransferases (DNMTs) including DNMT1, DNMT3a, and

DNMT3b. It is generally believed that DNMT3a and DNMT3b function as *de novo* methyltransferases, while DNMT1 is the primary maintenance enzyme. Recent evidence suggests that DNMT1 might also have *de novo* methylation activity. In the present study, we aimed to investigate the potential epigenetic modulation of DNMT1 on *Kcna2* gene in DRG under neuropathic pain conditions. DNMT1 is expressed exclusively in DRG neurons. Unilateral fifth spinal nerve ligation (SNL) increased the levels of DNMT1 mRNA and protein in the injured DRG. This increase was triggered by the activation of the transcription factor CREB. Blocking this increase via microinjection of adeno-associated virus expressing Cre into the injured DRG of DNMT1 floxed mice or via crossing these mice with a primary sensory neurons-specific line restored DRG *Kcna2* gene expression and function, reduced DRG neuronal excitability, and impaired SNL-induced neuropathic pain development. Moreover, DNMT1 bond to Kcna2 promotor regions and co-expressed with Kcna2 in individual DRG neurons. Given that Kcan2 is a key player in neuropathic pain genesis, DNMT1 is likely a new target for the treatment of neuropathic pain.

Disclosures: **L. Sun:** None. **X. Gu:** None. **S. Wu:** None. **L. Liang:** None. **B. Lutz:** None. **A. Bekker:** None. **Y. Tao:** None.

Poster

614. Molecular Mechanisms of Itch and Pain

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Support: NIH Grant NS072206

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Research Fellowship grant

Title: Epigenetic silencing of opioid receptors by G9a in in dorsal root ganglia after nerve injury

Authors: ***S. WU**[1], L. LIANG[1], X. GU[1], B. M. LUTZ[2], L. SUN[1], A. BEKKER[1], Y.-X. TAO[1]; [1]Anesthesiol., Rutgers, The State Univ. of New Jersey, Newark, NJ; [2]Anesthesiol., Rutgers Grad. Sch. of Biomed. Sci., Newark, NJ

Abstract: Downregulation of opioid receptors in the first-order sensory neurons of dorsal root ganglion (DRG) contributes to neuropathic pain genesis after nerve injury. Recent studies

suggested that epigenetic regulations such as DNA methylation and histone methylation may play roles in the downregulation of opoid receptors. We found that nerve injury induced the expression of histone methyltransferase G9a and its catalyzed repressive mark H3K9me2, which correlated with mu (MOR), kappa, and delta opioid receptor genes downregulation in the injured DRG neurons. Nerve injury-induced opioid receptors downregulation could be reversed by G9a knockdown and mimicked by G9a overexpression in the DRG. Chromatin immunoprecipitation assays demonstrated that nerve injury increased binding activitis of G9a and H3K9me2 to the promoter and 5'-untranslational regions of MOR gene. These data suggest that G9a is required for nerve injury-induced epigenetic silencing of opioid receptors. G9a inhibitors may serve as promising medications for the use as adjuvants with opioid in neuropathic pain management.

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Poster

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Support: R01NS094664

R01DA033390

U01HL117684

F31NS092310

Title: DNMT3a upregulation in DRG contributes to neuropathic pain development

Authors: ***B. LUTZ**[1], J.-Y. ZHAO[2,3], X. GU[1], L. LIANG[1], L. SUN[1], S. WU[1], J. FENG[3], A. BEKKER[1], E. J. NESTLER[3], Y.-X. TAO[1,2];
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Abstract: Neuropathic pain is a complex disorder in which current treatment options are often ineffective due in part to unexplained changes in gene transcription and translation in the first-order sensory neurons of dorsal root ganglion (DRG). Gene expression can be modified by DNA methylation. In this study, we reported that DNMT3a expresses exclusively in DRG neurons and

distributes predominantly in medium and large DRG neurons. *In vivo* studies revealed that the *de novo* methyltransferase DNMT3a (but not DNMT3b) expression increases at the levels of mRNA and protein in the neurons of the injured DRG at 3 and 7 days after spinal nerve ligation (SNL) in mice. No changes are seen after injection of complete Freund's adjuvant. Blocking the increase in DRG DNMT3a after SNL alleviates mechanical allodynia and thermal/cold hyperalgesia on days 3, 5, and 7 post-SNL. Additionally, overexpression of DNMT3a in DRG leads to the development of mechanical, thermal, and cold pain hypersensitivities. DNMT3a may participate in neuropathic pain development and be a novel target for advanced therapeutics.

Disclosures: **B. Lutz:** None. **J. Zhao:** None. **X. Gu:** None. **L. Liang:** None. **L. Sun:** None. **S. Wu:** None. **J. Feng:** None. **A. Bekker:** None. **E.J. Nestler:** None. **Y. Tao:** None.

Poster

614. Molecular Mechanisms of Itch and Pain

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Topic: D.02. Somatosensation: Pain

Support: NIH R01

Research Fellowship grants

Title: DNMT3a-mediated Kcna2 downregulation in DRG after peripheral nerve injury

Authors: ***X. GU**, J.-Y. ZHAO, L. LIANG, L. SUN, S. WU, J. FENG, K. MO, B. LUTZ, A. BEKKER, E. J. NESTLER, Y.-X. TAO;
Anesthesiol., NJMS, Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: The mechanisms of neuropathic pain development remain elusive; however it is known that changes in gene expression are associated with this disorder. DNA methylation regulates gene expression. Our pilot data indicate that nerve injury-induced increase in the expression of the *de novo* methyltransferase (DNMT3a) in the injured dorsal root ganglia (DRG) contributes to neuropathic pain development. However, the mechanism underlying this phenomenon is still unclear. We showed that the increase in DNMT3a is accompanied by a decrease in the expression of the voltage-gated potassium channel (Kcna2) in the injured DRG. *In vivo* studies show that overexpression of DNMT3a in mouse DRG leads to a decrease in Kcna2 mRNA and protein. Knockdown of DRG DNMT3a using a Cre-lox approach restored the expression of Kcna2 mRNA and protein on day 7 after spinal nerve ligation (SNL). Chromatin immunoprecipitation assays showed that the binding of DNMT3a to the Kcna2 promoter region

increases in the injured DRG on day 7 after SNL. This increase in binding is also accompanied by an increase in DNA methylation at the Kcna2 promoter. shRNA-mediated knockdown of DNMT3a abolished the increase in DNA methylation at the Kcna2 promoter. Electrophysiology studies showed an increase in DRG neuron excitability and a decrease in Kv current after DNMT3a overexpression in mouse DRG. These findings reveal that DNMT3a likely acts as an endogenous instigator of neuropathic pain genesis by epigenetically silencing DRG Kcna2.

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Poster

614. Molecular Mechanisms of Itch and Pain

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Topic: D.02. Somatosensation: Pain

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DA033390

Title: Contribution of the histone methyltransferase G9a in dorsal root ganglia to neuropathic pain

Authors: *L. LIANG[1], X. MIAO, 07103[2], X. GU, 07103[2], S. WU, 07103[2], H. SUN[3], B. LUTZ[2], A. BEKKER, 07103[2], E. J. NESTLER[3], Y. TAO, 07103[2];
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Abstract: The histone methyltransferase, G9a, methylates histone H3 on lysine residue 9 (H3K9) to predominantly produce dimethylation (H3K9me2), a dynamic histone methylation mark. This modification results in condensed chromatin and gene transcriptional repression. G9a is implicated in several biological processes including cell survival, cell death, drug addiction, stress responses, and other forms of behavioral plasticity through silencing specific gene expression. Whether G9a participates in neuropathic pain is unknown. Our immunohistochemistry staining showed that G9a co-expressed with NeuN in cellular nuclei but was not detected in GS-labeled cells. G9a was detected in all these three types of neurons including calcitonin gene-related peptide (CGRP, a marker for small DRG peptidergic neurons), isolectin B4 (IB4, a marker for small non-peptidergic neurons), and neurofilament-200 (NF200,

a marker for medium/large cells and myelinated A-fibers). Peripheral nerve injury increased the mRNA and protein expression of G9a and H3K9me2 in the injured DRG neurons. The fifth spinal nerve ligation (SNL)-induced pain hypersensitivities could be blocked by intrathecally administration of the G9a inhibitor BIX01294, by microinjection of adeno-associated virus (AAV)-Cre into the injured DRG of G9a flox mice, or in DRG G9a conditional knockout mice. Overexpression of G9a in DRG by microinjection of herpes simplex virus (HSV) expressing full-length G9a led to neuropathic pain symptoms. These data suggest that G9a is essential for SNL induced neuropathic pain and that G9a inhibitors may serve as promising adjuvants for neuropathic pain management.

Disclosures: **L. Liang:** None. **X. Miao:** None. **X. Gu:** None. **S. Wu:** None. **H. Sun:** None. **B. Lutz:** None. **A. Bekker:** None. **E.J. Nestler:** None. **Y. Tao:** None.

Poster

614. Molecular Mechanisms of Itch and Pain

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant NS94664

NIH Grant DA33390

Title: Contribution of the histone methyltransferase SUV39H1 in dorsal root ganglia and spinal cord dorsal horn to neuropathic pain

Authors: *Y. TAO[1], J. ZHANG[2], L. LIANG[2], S. WU[2], Q. MAO[2], M. XIONG[2], A. BEKKER[2]; [2]Anesthesiol., [1]New Jersey Med. School, Rutgers, Newark, NJ

Abstract: Peripheral nerve injury-induced alterations in the genes encoding receptors, ion channels, and enzymes in the dorsal root ganglion (DRG) and spinal cord dorsal horn are believed to participate in neuropathic pain genesis. Histone methylation gates gene expression. Whether the histone methyltransferase SUV39H1 contributes to neuropathic pain is unknown. We reported that SUV39H1 was detected in the neuronal nuclei of the DRG and dorsal horn. In the DRG, it was expressed predominantly in the small DRG neurons, in which it co-expressed with mu opioid receptor (MOR). The level of SUV39H1protein in both injured DRG and ipsilateral L_5 dorsal horn was time-dependently increased after the fifth lumbar spinal nerve ligation (SNL). SNL also produced an increase in the amount of SUV39H1 mRNA in the injured DRG. Intrathecal injection of chaetocin (a SUV39H1 inhibitor) as well as DRG or intraspinal

microinjection of SUV39H1 siRNA impaired SNL-induced pain hypersensitivity. DRG microinjection of SUV39H1 siRNA also restored MOR expression in the injured DRG. Our findings suggest that SUV39H1 contributes to nerve injury-induced pain hypersensitivity through gating MOR expression in the injured DRG. SUV39H1 inhibitors may be used with opioids in neuropathic pain management.

Disclosures: **Y. Tao:** None. **J. Zhang:** None. **L. Liang:** None. **S. Wu:** None. **Q. Mao:** None. **M. Xiong:** None. **A. Bekker:** None.

Poster

615. Neuropathic Pain I

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Topic: D.02. Somatosensation: Pain

Support: Helsinn HealthCare SA

Title: Ghrelin agonist HM01 attenuates oxaliplatin and cisplatin-induced neuropathy in murine models

Authors: ***B. S. SLUSHER**[1], K. M. WOZNIAK[1], Y. WU[1], E. POMMIER[1], Y. LIU[2], M. POLYDEFKIS[2], M. FARAH[3], R. RAIS[4], C. PIETRA[5];
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Abstract: Use of chemotherapeutic agents is associated with neuropathic toxicities which are often dose-limiting and have an impact on the life quality and survival of cancer patients. Ghrelin and ghrelin agonists, such as Anamorelin, have been found to be effective in preclinical and clinical studies of cancer-related cachexia. Ghrelin has also been shown to possess neuroprotective and neuropathic pain attenuating properties. In this study we evaluated the new PNS/CNS-penetrating ghrelin agonist HM01 in two experimental models of chemotherapy-induced neuropathy following nerve conduction velocity/amplitude, hyperalgesia, and footpad intra-epidermal nerve fiber density (IEFD). Oxaliplatin administered at its maximum tolerated dose in BALBc mice (6 mg/kg ip Q4Dx8), produced significant decreases in nerve conduction which were ameliorated by concomitant HM01 dosing (10 and 30 mg/kg PO daily). Specifically, oxaliplatin induced a significant decline in digital nerve conduction velocity (-10% $\pm$ 2.3%; $p < 0.05$) which was completely and significantly normalized by HM01 treatment. The oxaliplatin-induced digital amplitude deficit (-15% $\pm$ 7%; $p < 0.01$), although not statistically significant,

tended to also be improved by 10 and 30 mg/kg HM01 (19.8± 7.4% and 38.7± 10.9%, respectively; $p<0.05$ for 30 mg/kg dose). While there was no effect on sciatic nerve and dorsal root ganglia (DRG) morphology with this oxaliplatin regimen, a trend for reduction of IEFD in the footpads was observed (-15.1 ± 9.2%), which tended to be normalized by concurrent HM01 treatment. Interestingly, pharmacokinetic studies showed large accumulation of HM01 in DRG and sciatic nerve tissue (tissue penetration index of 18-19 and 9-12 fold versus plasma, respectively). In a separate cisplatin-induced neuropathy study, rats were dosed with cisplatin (0.5 mg/kg IP) and HM01 (3, 10 and 30 mg/kg PO) for several days. Cisplatin resulted in a significant decrease in food intake and weight gain, both of which were significantly prevented by concurrent HM01 treatment. Cisplatin also decreased the paw withdrawal threshold response to Von Frey filaments indicating development of mechanical hypersensitivity. Pretreatment with HM01 at doses of 10 and 30 mg/kg significantly reduced this hyperalgesia on multiple test days. Together these findings suggest a potential therapeutic use of the ghrelin agonist HM01 for treatment of both the nerve conduction impairments and painful hypersensitivity associated with chemotherapy-induced neuropathy.

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Poster

615. Neuropathic Pain I

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Izaak Walton Killam Memorial Scholarship

Title: Sex differences in pain and disease related outcomes in a mouse model of MS

Authors: ***K. A. MIFFLIN**[1], E. FRIESER[1], G. TENORIO[1], G. RAUW[2], G. BAKER[3], C. A. BENSON[1], B. J. KERR[4];
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Mental Hlth. Inst., [4]Departments of Pain Reserach and Anesthesiol. and Pharmacol., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Multiple Sclerosis (MS) is an inflammatory autoimmune and neurodegenerative disease. Although the primary symptoms of MS include the loss of sensory and motor function, many patients experience secondary symptoms such as chronic neuropathic pain. Recent work from our laboratory has explored voluntary exercise as a possible treatment for chronic pain in the mouse model commonly used to study MS: experimental autoimmune encephalomyelitis (EAE). These initial studies were carried out in female mice. We found that daily voluntary wheel running reduced pain at disease onset and also diminished dorsal horn microglial activation and T-cell infiltration. Voluntary wheel running was also shown to reduce oxidative stress in the spinal cord of female mice with EAE. Although exercise could delay the onset of clinical signs, there was no significant difference in the severity or progression of clinical signs once the disease was established. In the present study, we explored whether daily voluntary wheel running would also be effective at reducing nociceptive behaviour in male mice with EAE. Male mice were given access to a running wheel for 1 hour a day for 40 days. Surprisingly, voluntary wheel running did not reduce mechanical allodynia (tested with von Frey hairs) in males with the disease. We did find however, that unlike in females, daily wheel running significantly improved clinical scores in male mice with EAE. Direct comparison of inflammation, axonal injury, and oxidative stress in male and female mice with EAE revealed significant differences in the amount of T-cell infiltration, microglia and astrocyte reactivity, demyelination, and axon integrity in males and females with EAE. Male mice with EAE given daily access to running wheels had significantly less ongoing oxidative stress compared to all other groups. Taken together our results indicate that the inflammatory response generated in EAE is distinct between the sexes and its modulation by daily exercise can have sex specific effects on disease related outcomes.

Disclosures: K.A. Mifflin: None. **E. Frieser:** None. **G. Tenorio:** None. **G. Rauw:** None. **G. Baker:** None. **C.A. Benson:** None. **B.J. Kerr:** None.

Poster

615. Neuropathic Pain I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 615.03/JJ16

Topic: D.02. Somatosensation: Pain

Support: NHMRC Grant 631000

NHMRC Grant 1043933

Title: Aging alters inhibitory and excitatory synaptic input in the spinal cord superficial dorsal horn

Authors: *J. MAYHEW, R. J. CALLISTER, D. W. SMITH, B. A. GRAHAM;
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Abstract: The superficial dorsal horn (SDH) of the spinal cord is the first site in the central nervous system to process sensory processing from the body, and is essential for the segregation of sensory modalities, including touch, temperature, itch, and nociception. Disruption of this process can result in the emergence of aberrant sensory experiences such as allodynia and hyperalgesia. Importantly, altered pain states are more prevalent among the elderly, however the majority of preclinical studies on the underlying mechanisms of aberrant sensory processing are conducted in juvenile or young adult animals. Here we compare the spontaneous inhibitory and excitatory synaptic input to neurons in the SDH of young adult (3-4 months) and aged (28-32 months) mice. Briefly, male mice were deeply anaesthetized with ketamine (100mg/kg, i.p.) and decapitated. Sagittal spinal slices (300 um thick) were prepared from the lumbar cord. Patch clamp recordings were made with a CsCl-based (for inhibitory currents) or K-gluconate-based internal (for excitatory currents) from a holding potential of -70 mV. Because both GABA and glycine can mediate inhibition in the SDH recordings of mixed inhibition were first obtained (aged n =19, young n =9), before glycinergic inhibition was isolated by bath administration of bicuculline. Mixed sIPSCs from aged animals had similar amplitudes (-41.2 ± 5.5 pA vs -33.9 ± 4.5 pA), frequencies (0.4 ± 0.1 Hz vs 0.5 ± 0.2 Hz), and rise times (2.5 ± 0.2 ms v 2.5 ± 0.3 ms), but slower decay time constants (30.5 ± 2.4 ms vs 22.3 ± 3.6 ms). Subsequent application of bicuculline in a subset of recording (aged n = 8, young n = 8) dramatically reduced the frequency of glycinergic sIPSCs (0.07 Hz ± 0.02 vs 0.17 ± 0.4 Hz). In contrast, glycinergic sIPSC amplitude (-37.0 ± 10.8 vs -24.0 ± 3.8), rise time (2.3 ± 0.3 ms vs 2.5 ± 0.2 ms) and decay times (22.8 ± 5.0 vs v 21.9 ± 3.7) were similar in young and aged animals. Together, the slower decay of mixed sIPSCs and reduced frequency of glycinergic sIPSCs in aged recordings suggest a reduced role for glycinerigc inhibition in the aged SDH. For excitatory input (aged n = 38, young n = 21), sEPSCs from aged animals displayed reduced amplitudes (-15.3 ± 0.7 pA vs -25.0 ± 3.5 pA), but had similar frequencies (3.6 ± 1.0 vs 2.0 ± 0.6), rise times (1.24 ± 0.1 ms vs 1.23 ± 0.1 ms), and decay time constants (5.0 ± 0.3 vs 4.8 ± 0.5). Thus, the strength of excitatory input in the SDH diminishes with age. Together these age-related perturbations to excitatory and inhibitory synaptic transmission will impact baseline excitability of this region, upon which pathological disturbances such as allodynia and hyperalgesia are superimposed.

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Poster

615. Neuropathic Pain I

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Program#/Poster#: 615.04/JJ17

Topic: D.02. Somatosensation: Pain

Support: GRANT S268C

Title: Epigenetic regulation of chronic pain after mild traumatic brain injury

Authors: *D.-Y. LIANG, P. SAHBAIE, Y. SUN, X. SHI, A. MEIDAHL, T.-Z. GUO, P. LIU, D. C. YEOMANS, D. J. CLARK;
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Abstract: Chronic pain after traumatic brain injury (TBI) is very common, but the mechanisms linking TBI to pain and the pain-related interactions of TBI with peripheral injuries are poorly understood. In the current work we pursue the hypothesis that epigenetic changes induced by TBI support chronic pain sensitization. We used the rat lateral fluid percussion model to induce mild and moderate TBI. Some animals received hindpaw incisions as well. Mechanical allodynia was measured for up to eight weeks post-injury. Inhibitors of histone acetyltransferase (HAT) and histone deacetylase (HDAC) were used to probe epigenetic mechanisms. We followed serum markers including glial fibrillary acidic protein (GFAP), neuron-specific enolase 2 (ENO2) myelin basic protein (MBP) and S100β to gauge TBI injury severity. Our results showed that both mild and moderate TBI caused mechanical allodynia in the hindpaws of the rats lasting 3 or more weeks. Hindpaws contralateral to TBI showed more rapid and profound changes. The inhibition of HAT using curcumin 50 mg/kg s.c reduced mechanical sensitization while the HDAC inhibitor suberoylanilide hydroxamic acid 50mg/kg i.p. prolonged sensitization in the mild injury model rats. These agents did not systematically change serum markers in ways suggesting regulation of the degree of brain injury constituted a complete explanation for the observations. Immunohistochemical analyses of spinal cord tissue localized changes in the level of acetylation of the H3K9 histone mark to dorsal horn neurons. Taken together, these findings demonstrate that TBI induces persistent pain sensitization, and changes in spinal neuronal histone proteins may play an important role.

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Poster

615. Neuropathic Pain I

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Title: Ryr plays an important role through mitochondrial ros in hiv gp120-induced neuropathic pain in rats.

Authors: *K. GODAI[1,2], H. YI[2], S. LIU[2], K. TAKAHASHI[2], C.-H. LIU[2], Y. KANMURA[1], D. A. LUBARSKY[2], S. HAO[2];
[1]Anesthesiol. and Critical Care Med., Kagoshima Univ., KAGOSHIMA, Japan; [2]Anesthesiol., Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: The exact molecular mechanisms of human immunodeficiency virus (HIV) neuropathic pain are elusive. Ryanodine receptors (RyRs) are located in the endoplasmic reticulum (ER) membrane. ER stress and ROS are involved in neuropathic pain. In the present study, we investigated the relationship of RyRs and ROS in HIV coat glycoprotein gp120-induced neuropathic pain state in rats. HIV-related neuropathic pain was induced by repeated administration of intrathecal recombinant HIV gp120 in rats. Mechanical allodynia was assessed by measuring the threshold of paw-withdrawal response to graded mechanical stimuli using von Frey filaments and the up-and-down method. RyR expression was measured using western blots. Mitochondrial superoxide in the spinal dorsal horn was measured using MitoSox (a mitochondrial superoxide indicator) profile cells. Repeated administration of intrathecal recombinant HIV gp120 induced mechanical allodynia lasting for 4 weeks. Intrathecal gp120 increased the expression of RyR and upregulated the mitochondrial superoxide. Intrathecal administration of either RyR antagonist (dantrolene) or mitochondrial ROS scavenger Mito-Tempol increased mechanical threshold. Intrathecal dantrolene suppressed the upregulation of mitochondrial MitoSox profile cell number. These results demonstrated a substantial role of RyR through mitochondrial ROS in the HIV gp120-related neuropathic pain state, which provides a novel approach to treating HIV-related neuropathic pain.

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Poster

615. Neuropathic Pain I

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Topic: D.02. Somatosensation: Pain

Support: Korea Research Foundation (NRF) Grant 2012M3A9B6055414

Hugel Inc.

Title: Involvement of glial EphA4 receptor in development of trigeminal neuropathic pain in rats

Authors: ***M. KIM**[1], H. KIM[1], J. JU[1], J. SON[1], M. LEE[2], M. PARK[3], D. AHN[1];
[1]Sch. of Dent. Kyungpook Natl. Univ., Daegu, Korea, Republic of; [2]Dong-Eui Univ., Busan, Korea, Republic of; [3]Kyung-Woon Univ., Gumi, Korea, Republic of

Abstract: Glial-neuronal cross-talk plays a critical role in pain facilitation following peripheral nerve injury or in central sensitization that is characteristic of neuropathic pain. Ephrins and Eph receptors participate in the regulation of synapse formation through neuron-neuron and/or neuron-astrocyte interaction. However, it is still unknown whether Ephrin and Eph receptor-mediated cross talks at sites of glial-neuronal contact are involved in the central nociceptive processing associated with sensory abnormalities in persistent pain states. We hypothesized that changes in EphA4 receptor may play an important role in the development of neuropathic pain. For this purpose, the present study examined changes in EphA4 expression in the medullary dorsal horn and evaluated anti-nociceptive effects after blocking EphA4 pathway in rats with inferior alveolar nerve injury. Under anesthesia, the left lower second molar of male SD rats was extracted, followed by the placement of a mini dental implant to injure the inferior alveolar nerve. This injury produced mechanical allodynia along with the up-regulation of EphA4 and D-serine expression in the astrocytes and NR2 expression in the neurons of the medullary dorsal horn. The early treatment with EphA4-Fc, an EphA4 inhibitor, beginning on POD 0 for 3 days significantly attenuated mechanical allodynia and reduced up-regulated EphA4, D-serine and NR2 expression. Knockdown of EphA4 expression by intracisternal injection with EphA4 small interfering RNA also reduced mechanical allodynia produced by the inferior alveolar nerve injury. These results suggest that blocking EphA4 pathways in astrocytes produces attenuation of trigeminal neuropathic mechanical allodynia through a regulation of D-serine and NR2

expression in the medullary dorsal horn. These results provide that a modulation of the glial EphA4 signaling is a potentially important treatment strategy for trigeminal neuropathic pain.

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Poster

615. Neuropathic Pain I

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Program#/Poster#: 615.07/KK3

Topic: D.02. Somatosensation: Pain

Support: NIH Grant NS091759

Zilkha Family Discovery Fellowship in Neuroengineering

Title: Dissociated nociceptors generate irregular subthreshold fluctuations in resting membrane potential that are enhanced after spinal cord injury and contribute to spontaneous activity

Authors: ***M. A. ODEM**[1], R. M. CASSIDY[2], A. G. BAVENCOFFE[1], E. T. WALTERS[1]; [1]Integrative Biol. and Pharmacol., McGovern Med. Sch. At UTHealth, Houston, TX; [2]McGovern Med. Sch. at UTHealth, Houston, TX

Abstract: After thoracic (T10) spinal cord injury (SCI) in rats, primary nociceptors become hyperexcitable and generate spontaneous activity (SA) in their somata *in vivo* and after dissociation (Bedi et al., *J Neurosci*, 30:14870, 2010; Wu et al., *Pain*, 154:2130, 2013; Yang et al., *J Neurosci*, 34:10765, 2014). The increased incidence of SCI-induced SA correlates with behavioral hyperreflexia, and nociceptor hyperactivity is required for spontaneous pain. Wu et al. noted prominent depolarizing subthreshold fluctuations (SFs) of resting membrane potential (RMP) in nociceptors and suggested that these promote SA in C-fiber nociceptors after SCI. In A-fiber sensory neurons, sinusoidal subthreshold oscillations (SOs) were shown to be necessary for SA and to be enhanced by nerve injury (Amir et al., *J Neurosci*, 19:8589, 1999). We are using a novel quantitative method to characterize spontaneous SFs of RMP in nociceptors and their contributions to SCI-induced SA. Presumptive nociceptors are identified under whole cell current clamp 18-24 h after dissociation by an electrophysiological signature that reliably predicts capsaicin sensitivity in small DRG neurons. We have found that nociceptor SFs are irregular (non-sinusoidal) within the normal range of RMP in naïve and SCI conditions, confirming previous observations of their random nature (Study and Kral, *Pain*, 65:235, 1996). Whereas the incidence of irregular SFs reported in A-fibers is very low (3%; Amir et al., J

Neurosci, 22:1187, 2002), we have observed SFs in nearly all recorded nociceptors. SCI significantly increased the amplitude of the SFs in nociceptors exhibiting SA compared to silent nociceptors within the same range of RMP. The SCI-induced increase in amplitude of depolarizing SFs increases the likelihood that SFs reach firing threshold and thereby contribute to SA. Results thus far indicate that the basic mechanisms that generate irregular SFs in C-fibers differ substantially from those described for the sinusoidal SOs in A-fibers (Amir et al., Brain, 125:421, 2002). Interestingly, sinusoidal SOs can sometimes occur together with irregular SFs in the same C-fibers, especially when RMP is abnormally depolarized. Ongoing experiments are identifying ion channels important for generating SFs, as well as the cell signaling pathways that enhance SFs, SOs, and SA (see poster abstract by Bavencoffe, Odem, Dessauer, and Walters). Conditions that enhance nociceptor SFs and consequent SA may promote many forms of persistent pain.

Disclosures: M.A. Odem: None. **R.M. Cassidy:** None. **A.G. Bavencoffe:** None. **E.T. Walters:** None.

Poster

615. Neuropathic Pain I

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Topic: D.02. Somatosensation: Pain

Support: National Natural Science Foundation of China 31371121

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Natural Science Foundation of Jiangsu Province BK20140427

Title: Demethylated CXCR3 contributes to central sensitization and neuropathic pain

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[1]Nantong Univ., jiangsu, China; [2]Affiliated Hosp. of Nantong Univ., Nantong, China

Abstract: DNA methylation was recently implicated to be involved in the pathogenesis of chronic pain. Here we investigated how chemokine receptor CXCR3 is regulated by DNA methylation and its contribution to neuropathic pain induced by spinal nerve ligation (SNL) in mice. All animals used in this study were adult male ICR or C57BL/6 mice. Quantitative RT-PCR and ELISA showed that SNL increased *Cxcr3* mRNA and protein expression in the spinal cord. In situ hybridization combined with immunofluorescence staining further showed that

Cxcr3 was mainly expressed in spinal neurons. Meanwhile, the CpG (cytosine guanine dinucleotide) island in the *Cxcr3* gene promoter region was demethylated, and the expression of DNA methyltransferase 3b (DNMT3b) and its binding with *Cxcr3* promoter were downregulated in mice with neuropathic pain. Furthermore, SNL increased the binding ability of CCAAT/enhancer binding protein α (C/EBPα) with *Cxcr3* promoter and upregulated the expression of C/EBPα in dorsal horn neurons. Inhibition of C/EBPα by intrathecal siRNA attenuated SNL-induced pain hypersensitivity and reduced *Cxcr3* expression. Additionally, SNL-induced pain hypersensitivity was markedly reduced in *Cxcr3*$^{-/-}$ mice. Consistently, spinal inhibition of *Cxcr3* by shRNA or CXCR3 antagonist attenuated established neuropathic pain. On the other hand, CXCL10, the ligand of CXCR3 was increased in the spinal neurons and astrocytes after SNL. Intrathecal injection of CXCL10 induced CXCR3-dependent pain hypersensitivity. Finally, superfusion spinal cord slices with CXCL10 enhanced spontaneous EPSCs and potentiated NMDA- and AMPA-induced currents of lamina II neurons in a CXCR3-dependent manner. Collectively, our results demonstrated that CXCR3, increased by DNA demethylation and enhanced interaction with C/EBPα, can be activated by CXCL10 and facilitate excitatory synaptic transmission in neuropathic pain condition. Inhibition of CXCR3 signaling may provide a new therapy for neuropathic pain management.

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Poster

615. Neuropathic Pain I

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant NS091723

Craig H. Neilsen Foundation

Title: Spinal injury transforms how BDNF affects nociceptive sensitization and GABA function

Authors: *Y.-J. HUANG[1], K. H. LEE[2], J. W. GRAU[1];
[1]Neurosci., Texas A&M Univ., College Station, TX; [2]Neurobio., Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA

Abstract: In spinally transected rats, prior work has shown that both intrathecal (i.t.) BDNF and bicuculline (a $GABA_A$ receptor antagonist) administration exert a protective effect that counters

the learning impairment and enhanced mechanical reactivity (EMR) elicited by a peripheral irritant (capsaicin). In contrast, in intact rats, BDNF and bicuculline are thought to enhance capsaicin-induced nociceptive sensitization. We compared the effects of BDNF and bicuculline (i.t.) treatment in intact and spinally transected rats on capsaicin-induced EMR. Subjects received a complete spinal transection at T2 or sham operation. Twenty-four hours later, subjects received drugs (BDNF or bicuculline) or vehicle (i.t.) administration followed by peripheral treatment with capsaicin. We found both BDNF and bicuculline show opposite effects with or without SCI. BDNF and bicuculline both enhanced capsaicin-induced EMR in intact rats, but blocked it in transected rats. A cellular marker (ERK phosphorylation) of central sensitization exhibited the same pattern of results. These findings show that SCI alters how BDNF and GABA affect nociceptive processing within the spinal cord. BDNF is known to affect KCC2 expression, which determines GABAergic tone, and this effect may be modulated by SCI. To examine this possibility, we assessed KCC2 expression in BDNF treated intact and spinally transected rats. BDNF up-regulated KCC2 expression in transected rats, but down-regulated it in intact rats. Next, we tested whether BDNF also alters how a GABA agonist affects capsaicin-induced EMR. To test this, transected or sham-operated rats received BDNF or vehicle (i.t.) 24hrs after surgery. After BDNF treatment, subjects received either muscimol (a $GABA_A$ receptor agonist) or vehicle (i.t.) followed by capsaicin treatment. We found that BDNF treatment attenuated the muscimol-induced inhibition of EMR in intact rats. In transected rats, it had the opposite effect. These findings suggest that spinal injury transforms how BDNF affects GABA function and that this impacts the development of central sensitization.

Disclosures: Y. Huang: None. **K.H. Lee:** None. **J.W. Grau:** None.

Poster

615. Neuropathic Pain I

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant RO1 NS031680

Title: Electrophysiological evidence for the establishment of central sensitization in neuropathic mice.

Authors: *A. BITTAR[1], J. WANG[2], C. BAE[2], J.-H. LA[2], H. SHIM[2], J. CHUNG[2];
[1]Dept Neurosci & Cell Biol, Univ. Texas Med. Br., Galveston, TX; [2]UTMB, Galveston, TX

Abstract: Following nerve injury, aberrant afferent inputs lead to neuropathic pain. It is believed that long-term synaptic plasticity in the spinal dorsal horn underlie a state of increased excitatory but decreased inhibitory tones, leading to central sensitization and pain. However, long-term synaptic plasticity is seldom assessed in neuropathic animals. Rather, it is often induced in naïve intact spinal cords via a conditioning stimulus mimicking neuropathic conditions. Therefore, this study investigated whether long-term changes in the synaptic strength on two types of pain-relaying neurons were actually established in the spinal nerve ligation model (SNL). We hypothesized that long-term potentiation (LTP) would already be established in spinothalamic tract neurons (STTn), whereas long-term depression (LTD) would be established in GABAergic interneurons (GABAn) in the dorsal horn of SNL mice, leading to central sensitization. Long-term changes were assessed by comparing the following between naïve and SNL mice: 1) The frequency and amplitude of miniature-EPSCs (mEPSC) in STTn and GABAn, 2) The induction of synaptic plasticity following conditioning stimulus. Results showed that the frequency of mEPSCs is higher in SNL-STTn than in their naïve counterparts. However, the frequency of mEPSCs in SNL-GABAn is lower than that of naïve-GABAn. Furthermore, following low frequency stimulation, LTP was induced in the STTn of naïve mice but was occluded in the STTn of SNL mice. Similarly, LTD was induced in naïve-GABAn but occluded in SNL-GABAn. These results suggest that, in SNL mice, STTn are sensitized with increased excitatory synaptic activities, whereas GABAn are desensitized with decreased excitatory synaptic activities, leading to central sensitization and neuropathic pain.

Disclosures: **A. Bittar:** None. **J. Wang:** None. **C. Bae:** None. **J. La:** None. **H. Shim:** None. **J. Chung:** None.

Poster

615. Neuropathic Pain I

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant RO1 NS031680

Title: Capsaicin-induced secondary mechanical allodynia and hyperalgesia are mediated by different central sensitization mechanisms involving reactive oxygen species

Authors: *J.-H. LA, J. WANG, A. BITTAR, H. SHIM, C. BAE, J. CHUNG;
Dept. of Neurosci. and Cell Biol., Univ. of Texas Med. Br., Galveston, TX

Abstract: Intradermally injected capsaicin induces mechanical allodynia and hyperalgesia outside the injection site. Thus termed 'secondary', the two phenomena are attributed to 'central sensitization'. In humans, capsaicin-induced secondary mechanical allodynia (CSMA) and hyperalgesia (CSMH) are distinct in terms of their duration as well as their dependency on aberrant afferent discharges arising from the capsaicin injection site. This suggests that CSMA and CSMH are mediated by different central sensitization mechanisms. We tested this possibility in mice, focusing on the action of reactive oxygen species (ROS) that are shown to play a key role in central sensitization. Mice developed CSMA and CSMH to punctate stimuli after intradermal injection of capsaicin. As in humans, CSMA in mice lasted for a shorter time period than CSMH. In addition, unlike CSMH, CSMA was resolved by an anesthetic given at the capsaicin injection site. The ROS scavenger phenyl-*N*-tert-butylnitrone (PBN), administered prior to capsaicin injection, prevented the induction of CSMA, whereas it significantly shortened the duration of CSMH without preventing its induction. PBN treatment after capsaicin nearly abolished CSMA but only slightly attenuated CSMH. An intrathecal injection of the ROS donor, KO_2, induced both mechanical allodynia and hyperalgesia with the mechanical hyperalgesia outlasting the allodynia. These results suggest that the full activation of central sensitization mechanisms for CSMA and CSMH requires ROS. Once activated, the mechanism for CSMH is long-lasting and neither critically dependent on ongoing afferent inputs from the capsaicin injection site nor on the continuous presence of ROS. On the contrary, the presence of ROS and ongoing afferent inputs are indispensable for the development and maintenance mechanism for CSMA.

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Poster

615. Neuropathic Pain I

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant RO1 NS031680

Title: Iron chelation restores the GABAergic inhibitory function depressed in neuropathic pain

Authors: *H. SHIM, J.-H. LA, C. BAE, A. BITTAR, J. WANG, J. CHUNG;
Dept. of Neurosci. & Cell Biol., UTMB, Galveston, TX

Abstract: *Background:* One feature of neuropathic pain is a reduced gamma-aminobutyric acid (GABA)-ergic inhibitory function in the spinal cord. We previously demonstrated that oxidative stress induced both loss and dysfunction of GABAergic neurons (GABAn). Iron chelators have been known to reduce oxidative stress. However, the effect of iron chelation on neuropathic pain in relation to GABAn remains to be elucidated.
Objective: In this study, we investigated the effect of deferoxamine (DFO), an iron chelator, on spinal nerve ligation (SNL)-induced neuropathic pain in mice.
Methods: We examined the effects of intrathecally administered DFO, with and without the $GABA_A$ receptor antagonist bicuculline, on pain behaviors evoked by punctate mechanical stimulation in SNL mice. In electrophysiological studies, the effect of DFO on excitatory postsynaptic currents (EPSCs) was examined in GABAn using whole cell patch-clamp recordings in the mouse spinal cord slice.
Results: A single intrathecal injection of DFO significantly ameliorated SNL-induced mechanical allodynia. This analgesic effect of DFO was blocked by bicuculline. In electrophysiological recordings, tetanic afferent stimulation induced long-term depression (LTD) of EPSCs in GABAn. DFO prevented the induction of this LTD.
Conclusions: Taken together, these results suggest that iron chelation reduces neuropathic mechanical allodynia by restoration of GABAergic inhibitory function in the spinal cord.

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Poster

615. Neuropathic Pain I

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Topic: D.02. Somatosensation: Pain

Support: NIH grant RO1 NS091759

Title: Inhibition of EPAC signaling suppresses persistent spontaneous activity in primary nociceptors after spinal cord injury

Authors: *A. G. BAVENCOFFE, M. A. ODEM, C. W. DESSAUER, E. T. WALTERS; Integrative Biol. and Pharmacol., McGovern Med. Sch. At UTHealth, Houston, TX

Abstract: A majority of people with spinal cord injury (SCI) suffer from chronic neuropathic pain. In a rat T10 contusion model, nociceptors at and below the injury level exhibit chronic spontaneous activity (SA) generated in their somata in vivo and in vitro that persists for months

after SCI (Bedi et al., J Neurosci 30:14870, 2010). The nociceptor hyperactivity is correlated with chronic pain-related behaviors and is necessary for the maintenance of both reflex hypersensitivity and an operant measure of spontaneous pain (Yang et al., J Neurosci 34:10765, 2014). Recently we found that SCI-induced SA in dissociated nociceptors requires ongoing cAMP signaling in a macromolecular complex composed of A-kinase anchoring protein (AKAP150), adenylyl cyclase (AC5/6) and PKA (Bavencoffe, Li et al., J Neurosci. 36:1660, 2016). The importance of cAMP signaling raises the possibility that multiple downstream cAMP effectors might contribute to SCI-induced SA in nociceptors. We have begun to investigate the contribution of EPAC (Rap1 exchange protein activated by cAMP) to the maintenance of SA after SCI by examining the effects of an EPAC inhibitor, ESI-09. Neurons were isolated from DRGs L3 to L5 1-2 months after SCI and recorded 18-24 h after dissociation. Presumptive nociceptors were identified by characteristic electrophysiological properties we found to be a reliable signature for capsaicin sensitivity in small DRG neurons. As reported previously, SA incidence recorded in current clamp (I=0) was significantly higher in nociceptors from SCI rats (21 of 29 neurons) compared to nociceptors from naïve, uninjured rats (1 of 11 neurons). In the SCI group, incubation of sensory neurons with ESI-09 (5 μM) significantly reduced SA incidence. It also hyperpolarized resting membrane potential (RMP) and decreased the amplitude of subthreshold fluctuations of RMP (see poster abstract by Odem, Cassidy, Bavencoffe and Walters). Co-incubation with EPAC activator 8-pCPT-2-O-Me-cAMP-AM (10 μM) reversed the effects. In the naïve group, incubation of nociceptors with ESI-09 did not significantly change the low incidence of SA. However, ESI-09 produced a similarly large hyperpolarization of RMP in sensory neurons from the naïve and SCI groups. These observations indicate that ongoing EPAC signaling contributes to normal nociceptor excitability under our culture conditions and might also contribute to resting excitability of nociceptors in uninjured animals in vivo. An important question is whether SCI further enhances cAMP-EPAC signaling to produce nociceptor SA. Our results encourage the study of cAMP-EPAC signaling as a potential therapeutic target for chronic pain.

Disclosures: **A.G. Bavencoffe:** None. **M.A. Odem:** None. **C.W. Dessauer:** None. **E.T. Walters:** None.

Poster

615. Neuropathic Pain I

Location: Halls B-H

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Title: GPR151 in the spinal cord contributes to the pathogenesis of neuropathic pain

Authors: *Y. LU, T. YANG, B.-C. JIANG, W.-W. ZHANG, Y.-J. GAO;
Nantong Univ., Jiangsu, China

Abstract: G protein-coupled receptors (GPCRs), which consist of approximately 850 members, are the largest family of signaling proteins. Many of them are demonstrated to be involved in the development and maintenance of chronic pain. Using Gene array and Real-time PCR, we found that the expression of orphan G-protein coupled receptor 151 (GPR151) was dramatically increased in the spinal cord of adult male ICR mice after spinal nerve ligation (SNL). In this study, we investigated the role of GPR151 in SNL-induced neuropathic pain. Real-Time PCR showed that SNL induced persistent GPR151 upregulation in the spinal cord, which started 3 days after SNL and lasted for more than 21 days. Intrathecal injection of GPR151 siRNA or intraspinal injection of GPR151 shRNA lentivirus relieved SNL-induced neuropathic pain. GPR151 was mainly expressed in spinal astrocytes. Overexpression of GPR151 in primary cultured astrocytes increased the expression of phosphorylated extracellular signal-regulated kinase (pERK) and glial fibrillary acidic protein (GFAP, astrocytic marker). Intrathecal injection of GPR151 siRNA significantly reduced pERK expression in the spinal cord after SNL. Interestingly, SNL increased the binding ability of STAT3 (signal transducer and activator of transcription-3) with GPR151 promoter and the phosphorylation of STAT3. Inhibition of STAT3 by intrathecal injection of AG490 attenuated SNL-induced pain hypersensitivity and reduced GPR151 expression. Collectively, our results suggest that GPR151, upregulated in spinal astrocytes via the binding of STAT3 with its promoter, plays a crucial role in neuropathic pain through the activation of ERK signaling. Targeting GPR151 might provide a novel and promising approach to the treatment of neuropathic pain.

Disclosures: **Y. Lu:** None. **T. Yang:** None. **B. Jiang:** None. **W. Zhang:** None. **Y. Gao:** None.

Poster

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Topic: D.02. Somatosensation: Pain

Support: Korea Health technology R & D Project, Ministry of Health & Welfare, Republic of Korea (A120254)

Title: Signaling molecules involved in phosphatidylinositol-triphosphate (PIP3)-mediated ROS-induced AMPA receptor phosphorylation in the spinal dorsal horn of neuropathic rats

Authors: M. KO[1], S. JUNG[1], E. LEE[2], *J. W. LEEM[1];
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Abstract: Reactive oxygen species (ROS) in the spinal cord, which plays a crucial role in sensitization of dorsal horn neurons, has been implicated in neuropathic pain. ROS is an important mediator for regulating spinal AMPA receptor (AMPAR) phosphorylation to induce persistent pain. Phosphatidylinositol 3-kinase (PI3K) is involved in mediating inflammatory pain. In this work, we investigate a possible connection between PI-mediated signaling and AMPAR phosphorylation using nerve injury-induced and ROS donor-treated hyperalgesic rats. Mechanical hyperalgesia, induced by L5 spinal nerve ligation (SNL) or an intrathecal (i.t.) administration of a ROS donor (t-BOOH) and evaluated by measuring paw withdrawal threshold upon the application of von Frey hairs, was attenuated by pretreatment with either ROS scavenger alpha-phenyl-N-tert-butyl nitron (PBN) or PI3K inhibitor (wortmannin or LY294002). PIP3 levels, PI3K activity, and levels of oxidized inactive PI3-phosphatase (PTEN) were increased in the lumbar spinal dorsal horn of both SNL and ROS-treated rats. Such increases were attenuated by pretreatment with PBN or LY294002. An i.t. t-BOOH injection increased significantly phosphorylation levels of GluR1 at Serine-831 (GluR1-pS831) and at Serine-845 (GluR1-pS845) and GluR2 at Serine-880 (GluR2-pS880) compared to vehicle injection. All these elevated phosphorylation levels were attenuated by pretreatment with LY294002 or PIP3-dependent kinase (PDK) inhibitor (DCA), but not AKT inhibitor (MK-2206) or GSK3 inhibitor (AR-A014418). This increased phosphorylation was also attenuated for GluR1-pS831 by CaMKII inhibitor (KN-93), for GluR1-pS845 by PKA inhibitor (H-89), and for GluR2-pS880 by PKC inhibitor (GF109203X). GluR1 level in the membrane fraction and GluR2 level in the cytosolic fraction, sampled from lumbar spinal cord, were elevated in both SNL and i.t. t-BOOH-treated rats. The results suggest that ROS induce AMPAR phosphorylation through activation of PDK, but not of AKT/GSK3 signaling pathway. This PDK-mediated process may include phosphorylation of GluR1-S831 and GluR1-S845 by CaMKII and PKA, respectively, for the insertion of GluR1-containing AMPARs whereas phosphorylation of GluR2-S880 by PKC for endocytosis of GluR2-containing AMPARs at the postsynaptic zone, leading to central sensitization and thus neuropathic pain.

Disclosures: **M. Ko:** None. **S. Jung:** None. **E. Lee:** None. **J.W. Leem:** None.

Poster

615. Neuropathic Pain I

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Title: β-arrestin-2 regulates NMDA receptor function in spinal lamina II neurons and controls the duration of persistent pain and resolution of chronic pain

Authors: *G. CHEN[1], R.-G. XIE[1], Y.-J. GAO[2], Z.-Z. XU[1], L.-X. ZHAO[2], S. BANG[1], T. BERTA[1], C.-K. PARK[1], M. LAY[1], W. CHEN[3], R.-R. JI[1];
[1]Dept. of Anesthesiol. and Neurobio., Duke Univ. Med. Ctr., Durham, NC; [2]Inst. of Nautical Medicine, Co-Innovation Ctr. of Neuroregeneration, Nantong Univ., Nantong, China; [3]Dept. of Med., Duke Med. Ctr., Durham, NC

Abstract: β-arrestins are multifunctional scaffold proteins that regulate the desensitization of GPCRs including the mu opioid receptors. However, the unique role of β-arrestin in inflammatory and neuropathic pain has not been clearly investigated. Here we demonstrate an active role of β-arrestin 2 (Arrb2) in regulating spinal cord NMDA receptors (NMDAR) function and the duration of pain.
Intrathecal injection of the mu-opioid receptor agonist [D-Ala2, NMe-Phe4, Gly-ol5]-enkephalin (DAMGO) produces paradoxical behavioral responses: early-phase analgesia and late-phase mechanical allodynia; and notably, both phases are prolonged in Arrb2 knockout (KO) mice. Unlike the early-phase analgesia, the late-phase allodynia requires spinal cord activation of NMDAR. As expected, spinal administration of the NMDAR antagonist MK-801 reversed DAMGO-induced mechanical allodynia in WT and KO mice. Also, NMDA-induced currents in lamina IIo neurons of spinal cord slices were enhanced in DAMGO-treated WT mice at 24 h. Interestingly, spinal administration of NMDA induces GluN2B-dependent mechanical allodynia, which is prolonged in Arrb2-KO mice and conditional KO mice lacking Arrb2 in presynaptic terminals expressing Nav1.8. Loss of Arrb2 also increases the expression of synaptic GluN2B in lamina IIo neurons of spinal cord slices. Biotinylation experiment showed that the surface expression of Arrb2 was inversely correlated with that of GluN2B but not GluN2A in Hela cells, suggesting a specific interaction between Arrb2 and GluN2B. Loss of Arrb2 also results in prolongation of inflammatory pain and neuropathic pain and enhancement of GluN2B-mediated NMDA currents in spinal lamina IIo neurons. Finally, spinal over-expression of Arrb2 reverses

chronic neuropathic pain for several months.
In summary, using both loss-of-function (Arrb2-KO mice) and gain-of-function (Arrb2 over-expression) strategies, we demonstrate that Arrb2 controls the transition from acute to chronic pain via suppressing the activity of NMDAR/GluN2B in spinal lamina IIo neurons. Targeting spinal Arrb2 signaling may shed light on the development of new therapeutics for the prevention and treatment of chronic pain.

Disclosures: **G. Chen:** None. **R. Xie:** None. **Y. Gao:** None. **Z. Xu:** None. **L. zhao:** None. **S. Bang:** None. **T. Berta:** None. **C. Park:** None. **M. Lay:** None. **W. Chen:** None. **R. Ji:** None.

Poster

615. Neuropathic Pain I

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Topic: D.02. Somatosensation: Pain

Support: NINDS Grant R01NS079626-01

Title: Analgesic effect of intrathecal MSCs in nerve-injured rats is associated with changes in levels of multiple cytokines in CSF

Authors: ***G. FISCHER**[1,2], F. WANG[2], Z. LIU[2], X. BAI[2], H. YU[2], Q. HOGAN[2];
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Abstract: Neuropathic pain secondary to damage to peripheral nerves remains a serious, and unsolved, problem for a large number of patients. Recent studies have indicated that stem cells, particularly mesenchymal stem cells (MSCs) may have a constitutive effect on the evolution and maintenance of these phenomena. In the present study, we examined the effect of intrathecal administration of MSCs on established pain in animals which had undergone the tibial nerve injury (TNI) model. Specifically, MSCs were harvested from the femurs of young (4 to 5 weeks old) animals. Cells were cultured for the minimum time necessary to produce a culture of sufficient quality and quantity for injection. Animals showed a significant reduction in painful behavior to noxious stimulation after MSC administration, which was not reflected in animals receiving a control injection. This effect was long lasting, showing no sign of diminution for the duration of the study. Histological examination revealed that undifferentiated MSCs integrated into the white matter of the spinal cord and remained for the duration of the study. To illuminate the potential mechanisms mediating this analgesic effect, cerebrospinal fluid (CSF) was collected from animals and subjected to an antibody array to examine the effect of MSC administration on the expression levels of 67 cytokines in the CSF. Analysis showed that injury had a significant

effect on six cytokines: ICAM-1, IL-1β, IL-10, HGF, Idgcc4 (Nope), and Notch-1. Stem cell injection caused a significant change in five of these cytokines, ICAM-1, IL-1β, IL-10, HGF, and Idgcc4.

Disclosures: **G. Fischer:** None. **F. Wang:** None. **Z. Liu:** None. **X. Bai:** None. **H. Yu:** None. **Q. Hogan:** None.

Poster

615. Neuropathic Pain I

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Topic: D.02. Somatosensation: Pain

Support: NSFC Grant

Title: Role of LIM motif-containing protein kinases in chronic pain

Authors: ***X. YANG**[1], G. HE[1], L. CHEN[2], L. CHIANG[1], Z. JIA[3], W. LIU[2], Z. ZHOU[1]; [1]Inst. of Life Sciences, Southeast Univ., Jiangsu, China; [2]Jiangsu Key Lab. of Neurodegeneration, Nanjing Med. Univ., Nanjing, China; [3]The Hosp. for Sick Children, Univ. of Toronto, Toronto, ON, Canada

Abstract: LIM motif-containing protein kinases (LIMKs), a kind of serine/threonine kinase, are widely expressed in the central nervous system and are the regulator of Actin Depolymerizing Factor (ADF)/Cofilin. LIMK1/2 play important roles in the actin remodeling, which are critical to synaptic plasticity in the brain. To investigate the roles of LIMK1/2 in pain transmission and chronic pain formation in the spinal cord, we evaluated the behavioral changes and spinal cord synaptic transmission using LIMK1/2 double knock out (DKO) mice. Behavioral tests revealed that DKO mice have normal nociception properties, while compared to wild type (WT) mice, the hyperalgesia and alloydnia is significantly resisted in DKO mice in a battery of chronic pain models. Moreover, electrophysiological recordings showed altered excitatory synaptic transmission. Pharmacological inhibition of LIMK1/2 in WT mice significantly alleviated chronic pain phenotypes. These findings demonstrate that LIMKs are important in chronic pain in spinal cord via synaptic remodeling.

Disclosures: **X. Yang:** None. **G. He:** None. **L. Chen:** None. **L. Chiang:** None. **Z. Jia:** None. **W. Liu:** None. **Z. Zhou:** None.

Poster

615. Neuropathic Pain I

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Support: National Natural Science Foundation of China Grant 31371121

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Title: Chemokine pair CCL2/CCR2 regulates nerve injury-induced neuropathic pain and depression via increase of NR2B current in the nucleus accumbens of mice

Authors: *X.-B. WU, B. LIANG, P.-B. JING, Z.-J. ZHANG, Y.-J. GAO;
Nantong Univ., Jiangsu, China

Abstract: Symptoms including anhedonia, decreased motivation and depression are common affective features in patients with chronic pain. CC-chemokine receptor 2 (CCR2), a dominant receptor for monocyte chemoattractant protein-1 (MCP-1) has been the focus of attention because of its facilitative effects on nociceptive signal processing in chronic pain at peripheral nerve and dorsal horn. The nucleus accumbens (NAc) is an important area in mediating pain sensation and pain-related anxiety and depression. Whether MCP-1/CCR2 contribute to pain sensation and/or pain affect in the NAc remain unclear. In this study, we found that chemokine MCP-1 was increased in the neurons of NAc after spinal nerve ligation (SNL). CCR2 was also increased in NAc neurons after SNL. Inhibition of CCR2 by shRNA lentivirus in NAc shell attenuated SNL-induced pain hypersensitivity and depression-like behavior. Furthermore, whole-cell patch clamp recording of medium spiny neurons (MSNs) in NAc shell showed a significant increase in the function of NR2B-mediated currents after SNL, which was reduced by a selective CCR2 antagonist RS504393. Additionally, SNL increased the expression of phosphorylated NR2B (pNR2B), which was colocalized with CCR2 in the NAc. pNR2B upregulation by SNL was inhibited by intra-NAc injection of CCR2 shRNA lentivirus. Finally, Perfusing MCP-1 increased the NMDAR current. Intra-NAc MCP-1-expressing lentivirus increased pNR2B expression and induced pain hypersensitivity and pain-like behaviors. These results suggest that MCP-1/CCR2 signaling in the NAc is important in mediating the pain hypersensitivity and depression via NR2B following peripheral nerve injury.

Disclosures: **X. Wu:** None. **B. Liang:** None. **P. Jing:** None. **Z. Zhang:** None. **Y. Gao:** None.

Poster

615. Neuropathic Pain I

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Support: NIH Grant NS045594

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Title: Functional regeneration and reinnervation of the ligated spinal nerve in the rat spinal nerve ligation neuropathic pain model

Authors: *W. XIE, J. A. STRONG, J.-M. ZHANG;
Anesthesiol., Univ. Cincinnati Coll Med., Cincinnati, OH

Abstract: The spinal nerve ligation model (SNL; "Chung model"), in which the L5 (and sometimes L6) spinal nerves on one side are ligated, is a widely used rodent model of neuropathic pain. The model causes long-lasting thermal and mechanical hypersensitivity and allodynia of the hindpaw, and signs of spontaneous pain. Conceptually, the model was designed to allow separation of the roles of injured vs. uninjured sensory afferents in mediating pain; studies using this model almost always assume that the ligated spinal nerve does not regenerate and that any observed pain behaviors are mediated by neurons in the intact L4 DRG. While using this model, our dye tracing experiments showed apparent connections between the hindpaw and the ipsilateral L5 DRG neurons, leading us to conduct further experiments confirming that functional regeneration and target reinnervation of the L5 spinal nerve was occurring. The SNL model was implemented as in our previous studies: in Sprague Dawley rats of both sexes, the ventral ramus of the L5 spinal nerve on one side was ligated with 6-0 silk 2-3 mm distal to the DRG, then cut distal to the suture. In vivo, by day 14, a thin, clear, regenerated nerve could be observed emerging from the nerve stump proximal to the ligature, rejoining the distal segment of the ligated spinal nerve. By day 70 this regenerated nerve was thicker and whiter, more similar to the original structure. The neuronal tracer DiI injected into the paw labeled neuronal cell bodies (both NF200-positive and unmyelinated) in the L5 DRG (examined 5 -6 weeks after SNL). The long-lasting Dextran label injected into the proximal spinal nerve stump at the time of ligation could be observed in regenerated fibers in the paw skin as early as 21 days later. In vivo fiber recording from filaments teased from the L5 dorsal root and disconnected centrally verified that electrically stimulated axon potentials could conduct through the newly regenerated segment (observed in ~18% of filaments on day 14 and 80 - 100% from day 28 on). Signals from receptive fields stimulated in the periphery could also be detected conducting through the regenerated nerve; initially (day 14) most receptive fields detected were in the thigh and upper leg; by day 28 receptive fields in the knee, lower leg and paw began to be observed. We

conclude that functional regeneration and target reinnervation of the ligated spinal nerve is faster and more robust than has been assumed in this model, confounding some previous interpretations of studies with this model that assumed that ligation and suture would prevent reconnection of the L5 sensory neurons to the periphery.

Disclosures: **W. Xie:** None. **J.A. Strong:** None. **J. Zhang:** None.

Poster

615. Neuropathic Pain I

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Topic: D.02. Somatosensation: Pain

Support: NIH grant NS045594

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Title: Regeneration and target reinnervation of the injured nerve are associated with the persistence of neuropathic pain and spontaneous activity in rats

Authors: ***J.-M. ZHANG**, W. XIE, J. A. STRONG;
Dept Anesthesiol, Univ. of Cincinnati Col. of Med., Cincinnati, OH

Abstract: Abnormal spontaneous activity in sensory neurons is a common early feature of preclinical pain models, and blocking this activity blocks development of pain behaviors. Neuronal outgrowth during development also requires a period of spontaneous activity. The spinal nerve ligation model (SNL), in which the L5 spinal nerve on one side is ligated and cut, is a widely used rodent model of neuropathic pain. Recently (see related abstract) we have found that the spinal nerve undergoes functional regeneration and target reinnervation in this model. We hypothesize that regeneration is closely associated with spontaneous activity and pain. After implementing the SNL model in rats of both sexes, *in vivo* fiber recording (from filaments teased from the L5 dorsal root and disconnected centrally) showed a high incidence of spontaneous activity at day 14 or 28 (not including normal muscle spindle activity), and resection experiments showed the newly regenerated nerve was the primary source. The incidence declined gradually from day 28 to 70 (later times not examined). To inhibit nerve regeneration, we used local perfusion of the injury site via 14 day osmotic pump with semaphorin 3a (Sema 3a), an inhibitory axonal guidance molecule that collapses growth cones. In the SNL model, perfusion of the ligated spinal nerve starting immediately after injury reduced mechanical pain behaviors for the duration of the experiment (60 days) except for on day 1; and greatly reduced spontaneous

activity in fiber recording. Functional regeneration and target reinnervation were also impaired: receptive field responses conducting through regenerated nerve could only be detected in the thigh and upper leg, whereas without Sema-3a perfusion, receptive fields in the knee, lower leg, and paw could also be detected starting from day 28. Blocking activity at the injury site with 14 day local TTX perfusion also blocked mechanical pain behaviors at all time points (thru day 60, i.e., outlasting the drug perfusion time), and blocked reinnervation of more distal receptive fields. Our data suggest an association between nerve regeneration and its accompanying spontaneous activity, and pain behaviors.

Disclosures: J. Zhang: None. **W. Xie:** None. **J.A. Strong:** None.

Poster

615. Neuropathic Pain I

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AR062236-02

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Title: TNF and IFNβ comodulate tactile allodynia in a murine model of arthritis

Authors: *S. A. WOLLER[1], T. YAKSH[2], M. CORR[2];
[1]UCSD, LA Jolla, CA; [2]UCSD, La Jolla, CA

Abstract: Male C57Bl/6 (WT) mice develop transient inflammation in response to transfer of K/BxN serum and show a corresponding tactile allodynia (TA), which persists beyond the resolution of inflammation. Here, we examined the role of spinal cytokines, specifically TNF and IFNβ in the transition to a persistent pain state in males, which is mediated through Toll-like receptor (TLR) 4 signaling. Importantly, the contribution of TLR4 to pain states has been found to differ between male and female animals. Thus, our second aim was to examine whether female WT mice respond similarly to K/BxN serum transfer, and to examine whether TLR4 signaling associated spinal cytokines were involved in the development of TA in female animals.

In examining spinal cord TNF and IFNβ gene transcripts on d10 post K/BxN serum transfer, we found IFNβ transcripts decreased in WT mice (average fold change (AFC): 0.41) and were increased in *Tlr4*$^{-/-}$ mice (AFC: 18.84). In contrast, TNF transcripts increased in WT mice (AFC 1.33), and remained unchanged in *Tlr4*$^{-/-}$ mice (AFC 0.96). Next, we assessed the development of TA in male *Ifnar1*$^{-/-}$ and *Tnf*$^{-/-}$ mice. The inflammatory phase TA was attenuated in *Ifnar1*$^{-/-}$ mice (1.18g relative to 0.5g in WT males, $p < .05$); however these mice develop persistent TA while the late phase TA is reduced in *Tnf*$^{-/-}$ mice (1.29g relative to 0.74g in WT males, $p < .05$). Next, we examined female WT mice, and found they developed TA that was indistinguishable from males (0.72g), but, surprisingly, did *not* develop a persistent TA (1.36g, $p < .05$). The behavioral phenotype of female WT mice resembled that of *Tlr4*$^{-/-}$ males, and we assessed whether similar cytokine signaling contributed to the K/BxN TA phenotype. Both female *Ifnar1*$^{-/-}$ and *Tnf*$^{-/-}$ mice were indistinguishable from their male counterparts in terms of both ankle inflammation and TA. We then determined whether TNF and IFNβ transcripts change over time male and female WT mice. Males showed a general increase in spinal TNF mRNA expression relative to IFNβ, while females showed a higher ratio of IFNβ to TNF over time.
In another study, male WT mice were given IT anti-TNF antibody or IT IFNβ with no effect on TA. However, when *Tnf*$^{-/-}$ male mice were given IT IFNβ, we saw a persistent reversal in TA. *Ifnar1*$^{-/-}$ mice given IT anti-TNF antibody showed transient reversals in TA. Together, these results show persistent TA in WT male animals is associated with differential modulation of TNF and IFNβ. Female WT animals, however, fail to develop persistent TA, and show a recovery of spinal IFNβ transcription. The combination of genetic and pharmacological manipulations suggest that co-modulation of TNF and IFNβ may be necessary to prevent or reverse persistent TA.

Disclosures: **S.A. Woller:** None. **T. Yaksh:** None. **M. Corr:** None.

Poster

615. Neuropathic Pain I

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MOST 104-2745-B-002-001

Title: Plasticity changes of forebrain activity during neuropathic pain development in sciatic nerve injured rat

Authors: *T.-H. H. CHAO, J.-H. CHEN, R.-F. CHEN, C.-T. YEN;
Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Peripheral nerve damage frequently induces ectopic discharge in injured nerve fibers, which causes central sensitization and the development of neuropathic pain. Although there are many brain imaging studies of well-established chronic neuropathy, the involvement of the forebrain areas in the chronification process in the initiation phase is less studied. We hypothesize that ectopic barrage from the peripheral may cause sustained activation in the forebrain to initiate the plasticity involved in chronic neuropathic pain development.
In this study, we combined the advantages of different MRI methods, and demonstrated the transition of the brain activation changes during the neuropathic pain development in the spared nerve injury (SNI) model of the rat. We compared the brain activity during three different neuropathic pain development stage, including the moment of nerve injury using fMRI, and the 1st day and the 8th day after the neuropathic pain onset using manganese-enhanced magnetic resonance imaging (MEMRI).
Our main findings were: (1) Insular cortex (IC) and cingulate cortex (CC) showed sustained activation immediately after the SNI surgery. These sustained activations maintained at least 5 minutes throughout the whole fMRI scanning. (2) During the following days after the SNI surgery, we found consistently increased activation in ipsilateral IC. (3) Plasticity change of functional connectivity between ipsilateral IC, contralateral S1 and bilateral rostral anterior IC (RAIC) was established under the neuropathic pain condition. These results imply that IC may play an important role in neuropathic pain chronification.

Disclosures: **T.H. Chao:** None. **J. Chen:** None. **R. Chen:** None. **C. Yen:** None.

Poster

615. Neuropathic Pain I

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Support: Bio & Medical Technology Program through the NRF funded by the Ministry of Education, Science and Technology (2012M3A9C7050126, 2012M3A9B4028631), Republic of Korea

Title: Effects of analgesic substances on sensory and emotional pain-related behavior in neuropathic rats

Authors: *E. LEE, D.-W. KIM, J. LEEM, S. JUNG;
Dept. of physiology, college of medicine, Yonsei Univ., Seoul, Korea, Republic of

Abstract: The place-conditioning paradigm, which measures conditioned place avoidance, has been successfully used to analyze the affective component of pain. Although this behavioral test for analysis of emotional pain component is reliable for cases of acute pain, it is not applicable to chronic pain state including neuropathic pain.
The aims of the present study were 1) to establish the place avoidance paradigm applicable to emotional component of chronic pain, 2) to ensure the presence of emotional component of chronic neuropathic pain using the established behavioral testing protocol, and 3) to investigate the effects of several analgesic substances often used for pain management on the emotional component of neuropathic pain.
The place avoidance test, in which rats were able to avoid the compartment with a strong vinegar odor-producing 1% acetic acid and stay in the other compartment with cinnamon odor that provided masking effect, revealed that animals with an acute inflammatory pain induced by formalin injection into the hind-paw showed avoidance from the acetic acid odor compartment. This place avoidance behavior was prevented by bilateral lesions of the rostral anterior cingulate cortex (r-ACC) known to process emotional aspect of pain. From these data, we considered the place avoidance behavior as an expression of emotional pain component. For a rat model of neuropathic pain, sensory pain-related nociceptive behavior that was measured by hind-paw withdrawal test was significantly increased compared to sham-operated controls. The place avoidance test revealed that as compared to sham-operated rats, neuropathic animals stayed longer in a compartment with cinnamon odor than acetic acid odor. Bilateral lesions of r-ACC in neuropathic rats reversed the place avoidance behavior but not nociceptive behavior. However, bilateral lesions of the ventroposterior lateral nuclei of the thalamus that processes sensory component of pain reduced nociceptive behavior. In neuropathic rats, an intraperitoneal (i.p.) injection of amitriptyline (1.5 mg/kg) partly reversed nociceptive behavior whereas it completely blocked the place avoidance behavior. An i.p. gabapentin (50mg/kg) showed a significant decrease in both nociceptive and place avoidance behavior in neuropathic rats. On the other hand, i.p. memantine (20mg/kg) had a significant effect only on nociceptive behavior in neuropathic rats.
The results imply that the place avoidance paradigm used in the present study is proper for measuring emotional component of neuropathic pain and that the degree to which analgesic drugs contribute to alleviating each pain component is different.

Disclosures: **E. Lee:** None. **D. Kim:** None. **J. Leem:** None. **S. Jung:** None.

Poster

615. Neuropathic Pain I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 615.25/LL3

Topic: D.02. Somatosensation: Pain

Support: NIH Grant NS064341

NIH Grant DE14545

Title: Spinal nerve injury-induced aberrant tenascin-C upregulation contributes to pain hypersensitivity

Authors: *N. GONG[1], K.-W. LI[1], T. KWEON[1], B. VO[1], M. LI[1], N. ABDO[1], G. OREND[2], K. MIDWOOD[3], Z. LUO[1];
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Abstract: Neuropathic pain is a common disorder derived from nerve injuries and its mechanisms remain largely unknown. Our gene chip microarray analysis in dorsal root ganglion (DRG) neurons from a neuropathic pain model of unilateral spinal nerve ligation injury indicates that mRNA of tenascin-C, a large hexameric extracellular matrix glycoprotein, is increased in the injury side that correlates temporally with neuropathic pain state development. In this study, we examined the potential role of tenascin-C in promoting neuropathic pain development in the same neuropathic pain model of spinal nerve ligation injury. Our data indicated that tenascin-C protein levels were significantly upregulated in the injury side of DRG and dorsal spinal cord that temporally correlated with the development of neuropathic pain states. Blockade of injury-induced tenascin-C activity by intrathecal anti-tenascin-C antibodies or tenascin-C upregulation by siRNA oligonucleotides reversed established neuropathic pain states in rats post injury, although general knockout of the tenascin-C gene in mice could not prevent the development of neuropathic pain post injury. Intrathecal injection of recombinant tenascin-C proteins into naïve rats dose-dependently induced behavioral hypersensitivities, which could be reversed by intrathecal anti-tenascin-C antibodies. Thus, elevated spinal tenascin-C alone can lead to pain state development without the influence from other injury-induced factors. Since tenascin-C binds to different receptors, including toll-like receptor 4, epidermal growth factor receptors, integrin receptors, and annexin II receptors, we examined the potential receptor system(s) involved in tenascin-C mediated pain states. Antagonists or neutralizing antibodies against these potential tenascin-C receptors were injected intrathecally into tenascin-C injected rats with behavioral hypersensitivity, which was followed by behavioral testing. Our data indicated that only anti-annexin II receptor antibodies could reverse tenascin-C-induced pain states. Together,

these results support that injury-induced tenascin-C contributes to the development of neuropathic pain states through the annexin II receptors, and illustrate a pathological role of tenascin-C as an endogenous pain mediator.

Disclosures: **N. Gong:** None. **K. Li:** None. **T. Kweon:** None. **B. Vo:** None. **M. Li:** None. **N. Abdo:** None. **G. Orend:** None. **K. Midwood:** None. **Z. Luo:** None.

Poster

615. Neuropathic Pain I

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Topic: D.02. Somatosensation: Pain

Support: NSERC

Title: Using two-photon calcium imaging to detect antidromic spikes evoked by presynaptic $GABA_A$ receptor activation in primary afferent neurons

Authors: ***P. TAKKALA**[1], S. A. PRESCOTT[2];
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Abstract: Presynaptic GABAergic input on primary afferent neurons is generally inhibitory but can evoke spikes under certain pathological conditions. Antidromic conduction of those spikes can lead to peripheral release of inflammatory mediators, resulting in neurogenic inflammation. Due to the high intracellular chloride concentration in primary afferent neurons, activation of $GABA_A$ receptors on their central terminals causes primary afferent depolarization (PAD). PAD normally inhibits spike propagation and synaptic release via shunting effects and sodium channel inactivation but its alteration can lead to PAD-induced spiking. We have previously described the conditions under which PAD can evoke spikes, in particular, simultaneous intracellular chloride accumulation and altered spike initiation properties (i.e. enhanced excitability). This combination of changes can arise in damaged peripheral nerves via enhanced function of the Na-K-Cl cotransporter, NKCC1, and downregulation of K_v1-type potassium channels, respectively. However, it remains unclear if these requirements are met in central axon terminals to generate action potentials that can then propagate to the periphery.
To image spiking in neurons with intact central terminals, we first validated our technique for optical detection of GABA-evoked spikes in cultured neurons. Using dissociated dorsal root ganglion (DRG) neurons from GCaM6f+ mice, we sought to determine the proportion of neurons in which puffed GABA can evoke spikes after enhancing NKCC1 function and/or blocking K_v1 channels. As predicted, we found a significantly greater proportion of neurons

displayed GABA-evoked spike initiation only when the two experimental manipulations were combined. Then, using an ex vivo preparation in which the dorsal root ganglion is still attached to the spinal cord, two-photon imaging at the DRG revealed that GABA applied to axon terminals in the spinal dorsal horn evoked antidromically propagating spikes only under the conditions described previously. By acutely reproducing the changes required for central terminal GABA-evoked induction of action potentials in peripheral nerves, we have shown that, like in the soma, PAD-induced spiking in central axon terminals requires joint changes in the chloride reversal potential and excitability.

Disclosures: P. Takkala: None. **S.A. Prescott:** None.

Poster

615. Neuropathic Pain I

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 615.27/LL5

Topic: D.02. Somatosensation: Pain

Title: Purinergic dose response analysis in mouse dorsal root ganglion neurons

Authors: R. W. HUGHEN, K. J. VOLL, J. C. JENSEN, L. R. KEMKER, A. R. LIGHT, *J. ZHANG;
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Abstract: Adenosine triphosphate (ATP) is the ubiquitous energy source for metabolism. Importantly, ATP is also an extracellular signaling molecule involved in many processes including pain and fatigue. ATP is released into the interstitium through channels, transporters and exocytosis. Physiological or pathophysiological conditions such as muscle exercise or tissue damage significantly increase the extracellular ATP concentration. Levels of extracellular ATP are detected by a group of purinergic receptors (P2 receptors), some of which are highly expressed in nociceptors (e.g., P2X3 and heteromeric P2X2/3 receptors). Our lab observed that ATP is one of the most potent metabolites in activating muscle-innervating dorsal root ganglion (DRG) neurons. To further pursue an ATP dose response curve for the DRG neurons, we did FURA2 calcium imaging experiments. DRGs were collected from male C57Bl mice that had hindlimb skeletal muscles injected 2 weeks previously with the retrograde fluorescent dye (2*Z*)-2-[(*E*)-3-(3,3-dimethyl-1-octadecylindol-1-ium-2-yl)prop-2-enylidene]-3,3-dimethyl-1-octadecylindole (Di-I). Primary neural cultures were made two weeks after the Di-I injection to allow the dye to be transported to the cell body in the DRGs. For each imaging experiment, Di-I labeled neurons (i.e., muscle-innervating cells, usually over 20% of the image field) are identified and analyzed separately from non-labeled neurons, as described before (Light et al

2008). Di-I labeled cells were also collected individually using a micropipette for RNA-Seq analysis. In the calcium imaging experiments, we challenged the cells with serial log concentrations ATP from 1nM - 10mM. We also investigated the ATP degradation/hydrolysis rate in aqueous solution. We found 50% degradation of ATP occurred about 20 min after it was dissolved in solution. Because ATP hydrolyzes predominantly to adenosine diphosphate (ADP), we also investigated the ADP dose response curve of the same concentration range to understand possible inhibitory/antagonistic effects of ADP on DRG neurons. We challenged the cells with ATP and ADP separately during the same experiment to see if different cells responded to ATP than ADP at a given concentration. We used specific antagonists 2',3'-*O*-(2,4,6-Trinitrophenyl) ATP (TNP-ATP) and pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) to identify specific P2 receptors being activated. Our RNA-Seq data indicated at least 10 different P2 receptors on individually collected DRG neurons capable of responding to ATP. Of these, P2X3,4,5,7 and P2Y1,2, and 14 are most likely the major contributors to the activation of DRG neurons by ATP/ADP.

Disclosures: R.W. Hughen: None. **K.J. Voll:** None. **J.C. Jensen:** None. **L.R. Kemker:** None. **A.R. Light:** None. **J. Zhang:** None.

Poster

615. Neuropathic Pain I

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HHMI medical felloship (SAL)

Title: Mu and delta opioid receptors on muscle innervating group III/IV afferent neurons modulate muscle fatigue and ache

Authors: J. ZHANG[1], *A. R. LIGHT[3], R. W. HUGHEN[3], H. HUANG[1], W. TANG[2], J. C. JENSEN[2], S. A. LOW[4], G. SCHERRER[5];
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Abstract: We used double mutant mice with both a delta opioid receptor reporter linked to green fluorescent protein (DOReGFP) and a Mu opioid receptor reporter linked to mcherry (MORmcherry) to determine the functional organization of opioid receptors on Group III and IV neurons that signal muscle pain and fatigue, and also are the afferent arm of the exercise pressor reflex. We used DiI (1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate) injected into the gastrocnemius muscle of seven, 30 day old, ketamine/zylazine anesthetized mice to retrogradely label dorsal root ganglion (DRG) neurons innervating this skeletal muscle. To determine the activation of DiI labeled neurons by exercise induced metabolites (a combination of ATP, lactate and protons) we used calcium imaging with Fura 2 as in Light et al, 2008. Our results from 815 cells (114 of which were labeled with DiI from 7 different mice (both male and female) confirmed the findings of Scherrer et al, 2009 and Bardoni et al, 2014 that about 31% of all DRG neurons express mu receptors, and 12% of all DRG neurons express delta receptors. Several differences between opioid receptors on all DRG neurons vs. opioid receptors on DiI labeled (muscle innervating neurons) were found. 1. More DiI labeled neurons (66%) expressed opioid receptors. 2. Delta receptors were much more common in DiI labeled neurons (40%) than in non muscle labeled neurons (7%); mu receptors were also more common (43% in DiI labeled neurons vs 29% in neurons not labeled with DiI). 3. As in Light et al, 2008 and Jankowski et al, 2013 about 41% of DiI labeled neurons responded to metabolites and half of these responded to low levels of metabolites (likely non-nociceptive) while the other half responded to high levels of metabolites (likely nociceptive). 4. Opioid receptors were very common on muscle labeled neurons responding to metabolites (77%) with 44.7% of these being mu receptors and 42.6% being delta receptors. 5. Delta receptors were more common in low metabolite responding neurons (50% delta, 33% mu), while mu receptors were more common in high metabolite responding neurons (56% mu, 32% delta). Thus, mu and delta opioid receptors likely play a larger role in regulating muscle innervating neurons than regulating other types of afferent neurons (e.g.,skin, viscera). Also delta opioid receptors likely play a larger role in modulating low metabolite responding neurons (which signal fatigue and activate the exercise pressor response) while mu receptors play a larger role in modulating high metabolite responding neurons (which signal ache).

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Poster

615. Neuropathic Pain I

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Research, Innovation, and Pilot Funding Program at CCHMC

Title: Neonatal growth hormone treatment blocks the priming effects of early life injury on adult hypersensitivity to inflammation

Authors: *Z. K. FORD[1], X. LIU[1], B. KATRAGADDA[1], K. J. GREEN[1], R. C. HUDGINS[1], M. P. JANKOWSKI[1,2];
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Abstract: Exposure to injury as a neonate has the potential to prime the nervous system to subsequent re-injury later in life, which can have lasting effects on nociceptive processing in adulthood. Thus, strategies designed to mitigate the long term consequences of early life insult on adult hypersensitivity to peripheral injuries are of utmost importance. We have previously shown in a murine inflammatory pain model that injecting 3% carrageenan into the hairy hindpaw skin of neonatal mice produces a transient, but target-specific, decrease in growth hormone (GH) levels, which corresponded to observed mechanical and thermal hypersensitivity both *in vivo* and when assessing the response properties of cutaneous afferents with our *ex vivo* somatosensory system recording preparation. Systemic treatment with low dose GH blocked inflammation-induced hypersensitivity both behaviorally and electrophysiologically, while restoring the cutaneous quantities of GH back to naïve levels. In order to explore if GH was also proficient in preventing the priming effects of early life insult on adult hypersensitivity to cutaneous re-injury, we treated postnatal day 7 (P7) neonates with GH or vehicle at the time of cutaneous inflammation and re-inflamed the periphery 4-5 weeks later. For comparison, a third cohort of mice received vehicle as neonates, but no cutaneous inflammation. We then assessed DRG gene expression and mechanical and thermal responsiveness in these groups 1-28d post adult inflammation. We found that double-inflamed mice were hypersensitive to both mechanical and heat stimuli for up to 3 weeks, while GH-treated mice returned to baseline levels within one week, similar to animals that only received carrageenan injection as young adults. Corresponding changes in mechanically and thermally sensitive receptors/channels were also found in our groups after adult inflammation in the DRGs. Results indicate that a GH treatment in neonatal mice may have the ability to block the priming effects of early life insult on adult hyper-responsiveness to both mechanical and thermal stimuli after cutaneous re-injury. This data may provide a potential therapeutic strategy to prevent the transition from acute to chronic pediatric inflammatory pain.

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Poster

616. Neuropathic Pain II

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Topic: D.02. Somatosensation: Pain

Support: R21 AA023051

R01 DA018156

T32-AA014127

P50-AA022534

Title: Prenatal alcohol exposure has enduring effects on spinal glial-immune responses following minor peripheral nerve damage leading to enhanced neuropathic touch hypersensitivity

Authors: *J. J. SANCHEZ[1], M. S. SUN[2], S. DAVIES[2], D. D. SAVAGE[2,3,4], E. D. MILLIGAN[2,5];
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Abstract: A hallmark of neuropathic pain is increased sensitivity to light touch, clinically known as mechanical allodynia. Glial cells (astrocytes and microglia) contribute to ongoing allodynia in various animal models such as a localized sciatic nerve chronic constriction injury (CCI), a classic rat model of peripheral neuropathy. Released glial proinflammatory cytokines like interleukin-1β (IL-1β) that activate IL-1 receptors on pain neurons and other nearby glia are critical in mediating allodynia. Patients with fetal alcohol spectrum disorder experience sensitivity to light touch and have aberrant immune responses. Studies in animal models of prenatal alcohol exposure (PAE) demonstrate increased withdrawal responses to mildly noxious thermal stimuli. Therefore, we examined whether PAE causes a lifelong enhancement of allodynia and spinal glial activation in response to standard-CCI (4-suture loose ligation) or a minimal CCI (1-suture) model that does not produce allodynia in non-PAE animals.
Methods: Saccharin (Sac controls) or PAE dams consumed 5% ethanol (v/v) four hours each day throughout pregnancy. Adult male offspring (4 mo. or 1 yr.) were assessed for hindpaw light touch withdrawal thresholds (Von Frey test for allodynia) prior to sham or CCI surgery and after surgery. On day 28 after surgery, 4 mo. rats received intrathecally (i.t.) either vehicle or BIRT-377, an antagonist to lymphocyte function-associated protein 1(LFA-1), which is expressed by spinal microglia and immune cells during their activation and migration. Allodynia was re-assessed daily for 4 days, followed by transcardial saline/paraformaldehyde perfusion for immunohistochemical detection of lumbar dorsal spinal cord glial activation markers.

Results: 4-suture CCI enhanced allodynia in 4 mo. and 1 yr. PAE rats compared to controls, and 1 suture CCI induced unilateral allodynia in both 4 mo. & 1 yr. old PAE rats, but not in Sac controls. In 4 mo. 4-suture CCI PAE rats, bilateral astrocyte and microglial activation was highest while 1 yr. 4 suture CCI PAE revealed elevated ipsilateral astrocyte and microglial activation. Curiously, 1 yr. 1-suture CCI PAE did not induce increased microglial activation. Finally, in 4 mo. old PAE rats, i.t. BIRT-377 caused complete reversal of allodynia while corresponding astrocyte and microglial activation remained elevated. Ongoing studies are examining the effects of BIRT-377 on spinal glial & IL-1β responses.
Conclusions: Results indicate that PAE causes lifelong enhancement of spinal glial-immune reactivity that may underlie a susceptibility to developing chronic neurological disorders including neuropathies in humans.

Disclosures: **J.J. Sanchez:** None. **M.S. Sun:** None. **S. Davies:** None. **D.D. Savage:** None. **E.D. Milligan:** None.

Poster

616. Neuropathic Pain II

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Title: Involvement of CD137L in peripheral nerve injury-induced neuropathic pain

Authors: ***L. CAO**, J. T. MALON, R. LEEMING;
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Abstract: CD137L, also known as 4-1BBL, is a co-stimulatory molecule involved in many immune functions. CD137L-mediated signaling can promote monocyte/macrophage proliferation, migration, and production of proinflammatory factors; however, CD137L-mediated microglial responses have not been studied extensively. Here, we sought to investigate the role of microglial CD137L in the development of peripheral nerve injury-induced neuropathic pain using a murine model, spinal nerve L5 transection (L5Tx). First, compared to wild type (WT) controls, B6_CD137L knockout (KO) mice displayed significantly decreased L5Tx-induced mechanical and heat hypersensitivity starting at day 3 post-L5Tx until day 35 post-L5Tx when the experiment was terminated. To determine the role of microglial CD137L, an anti-CD137L neutralizing antibody (Ab, clone TKS-1) was intrathecally injected into WT BALB/c mice daily from day 0 (before surgery) to day 7 post-L5Tx. The neutralizing Ab significantly reduced

L5Tx-induced mechanical hypersensitivity but not the heat hypersensitivity. Further, we evaluated the microglial M1 vs M2 responses following L5Tx in both CD137L KO and WT mice via qRT-PCR and immunohistochemistry. Preliminary results suggest there may be less sustained M1 and M2 responses in CD137L KO mice compared to WT mice. Altogether, our data indicate that spinal cord CD137L contributes to the maintenance of peripheral nerve injury-induced neuropathic pain-like behaviors, which may be in part due to a reduced activation of spinal cord microglial cells.

Disclosures: **L. Cao:** None. **J.T. Malon:** None. **R. Leeming:** None.

Poster

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Support: the Ministry of Science, ICT, and Future planning Grant (2014M3C1B2048632)

Title: Glial inhibition through single transcutaneous electrical nerve stimulation reduces knee osteoarthritic pain in rats

Authors: *S.-C. HAHM[1,2], E. SONG[1,2], H. JEON[1,2], Y. YOON[3], J. KIM[1,2];
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Abstract: Transcutaneous electrical nerve stimulation (TENS), noninvasive intervention, is commonly used for treatment of acute or chronic pain in several pathologic conditions, such as osteoarthritis (OA). However, analgesic effects of TENS and its mechanisms are still unclear. The aim of this study is to investigate the efficacy of single TENS for pain treatment in rats with knee OA and its mechanisms related to glial cell inhibition. OA was induced by injecting monosodium iodoacetate (4 mg / 50 μl) into the synovial space of the right knee joint. High frequency (HF)-TENS (100 Hz), low frequency (LF)-TENS (4 Hz), or sham-TENS with 100 μs pulse duration and sub-motor threshold was applied for 20 minutes on the ipsilateral knee joint. Knee bend score (KBS), paw withdrawal threshold (PWT) and weight bearing were measured before and after TENS application. Immunohistochemistry for microglia and astrocyte was performed in the spinal L3-5 segments. In order to test the effects of glial cells inhibition on OA pain, minocycline, L-α-aminoadipate (LAA) or artificial cerebrospinal fluid (aCSF) was injected intrathecally. KBS and PWT were measured before and after drug delivery. Both HF- and LF-

TENS significantly decreased KBS and increased PWT, but did not recover the decreased weight load of ipsilateral side during locomotion. Both HF- and LF-TENS inhibit activated microglia in L3-5 compared to sham-TENS. Spinal astrocyte significantly reduced in L3-5 by LF-TENS and in L3 by HF-TENS. Both minocycline and LAA decreased KBS and increased PWT compared to aCSF. These results suggest that both HF- and LF-TENS alleviate OA pain in rats through inhibition of activated microglia and reduction of astrocyte expression in the spinal cord.

Disclosures: **S. Hahm:** None. **E. Song:** None. **H. Jeon:** None. **Y. Yoon:** None. **J. Kim:** None.

Poster

616. Neuropathic Pain II

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R01DE021996

T32DA007234

T32GM008471

Title: Activation of microglial C3aR1 by the VGF-derived peptide TLQP-21 may contribute to nerve-injury induced hypersensitivity

Authors: ***J. L. COOK**, M. RIEDL, K. KITTO, C. HONDA, C. FAIRBANKS, L. VULCHANOVA;
Univ. of Minnesota, Minneapolis, MN

Abstract: VGF is a neuropeptide precursor whose expression is rapidly and robustly increased in dorsal root ganglion and dorsal horn neurons following peripheral nerve injury. We have demonstrated that the VGF-derived peptide TLQP-21 contributes to both the development and maintenance of hypersensitivity after peripheral nerve injury and inflammation, and that intrathecal administration of TLQP-21 results in thermal hyperalgesia. The receptor for TLQP-21 was found to be the complement
3a receptor C3aR1. We have determined that C3aR1 appears to mediate the spinal effects of TLQP-21, as TLQP-21-evoked thermal hyperalgesia is inhibited dose-dependently by the C3aR1 antagonist SB290157. We aimed to examine whether the spinal effects of TLQP-21 were mediated by a direct interaction with this receptor, rather than activation of the complement

pathway and generation of C3a. It was found by Cero et al that the terminal arginine of TLQP-21 is required for activation of C3aR1, and that an arginine-to-alanine (R21A) substitution disrupted this. We generated a modified TLQP-21 peptide, with the R21A substitution (TLQP-R21A). When injected intrathecally, TLQP-R21A (1 nmol) demonstrated an attenuated thermal hyperalgesia compared to TLQP-21 (1 nmol), indicating that the spinal effect of TLQP-21 is largely mediated by activation of C3aR1. To examine potential cell types that respond to TLQP-21, we performed calcium imaging in cultured primary microglia. Stimulation with TLQP-21 evoked calcium mobilization in the cultured primary microglia, and this failed to occur in cultures prepared from C3aR1 knockout mice, indicating that the effect of TLQP-21 requires C3aR1. To visualize the interaction of TLQP-21 with C3aR1 in cultured primary microglia, we employed Click-iT chemistry. Cultures were incubated with an azide-modified TLQP-21, and then through a copper-catalyzed Click-iT reaction, were stained with a fluorescently-labeled alkyne. Preliminary results show a staining pattern that is consistent with receptor internalization, which provides further evidence of direct activation of C3aR1 by TLQP-21 in microglia. To determine whether C3aR1 upregulation after nerve injury may contribute to the involvement of TLQP-21 in hypersensitivity, we examined changes in receptor expression using quantitative real-time PCR. We found that C3aR1 mRNA expression is increased in the ipsilateral lumbar spinal cord compared to the contralateral side in spared nerve injury animals, and not in sham controls. This points to a potential mechanism for increased signaling by TLQP-21 following injury.

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Poster

616. Neuropathic Pain II

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2013-R1A2A2A01067248

Title: IKK/NF-κB-dependent satellite glia activation induces spinal cord microglia activation and neuropathic pain via ganglioside-TLR2 signaling

Authors: ***H. LIM**[1], H. LEE[1], K. NOH[1], B. YOU[1], J. OH[1], H. MOK[2], B. KIM[3], S. BACK[4], J.-S. PARK[1], K. KIM[2], R. SCHNAAR[5], M. KARIN[6], S. LEE[1];

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Abstract: Increasing evidence supports that both microglia and satellite glial cell (SGC) activation play a causal role in neuropathic pain development after peripheral nerve injury, yet the activation mechanisms and their contribution to neuropathic pain remain elusive. To address this issue, we generated *Ikkβ* conditional knockout mice in which IKK/NF-κB-dependent proinflammatory SGC activation is abrogated selectively in dorsal root ganglion (DRG). In these mice, nerve injury-induced proinflammatory gene expression and macrophage infiltration into the DRG were severely compromised. Likewise, nerve injury-induced spinal cord microglia activation and pain hypersensitivity were significantly attenuated in these mice compared to control mice. However, macrophages recruited into the DRG *per se* have minimal effects on spinal cord microglia activation suggesting a causal effect of SGC activation on spinal cord microglia activation. In an effort to elucidate the molecular mechanisms, we found that IKK/NF-κB-dependent SGC activation induced *St3gal2* expression in sensory neurons and subsequently leads to aberrant increase in ganglioside GT1b in afferent axons in the dorsal horn. In the spinal cord, GT1b functions as an endogenous agonist of toll-like receptor 2 (TLR2) to activate microglia and thereby induces pain central sensitization. Taken together, we present a novel mechanism for spinal cord microglia activation in nerve injury-induced neuropathic pain that is dependent on SGC activation, GT1b increase in the dorsal horn, and activation of microglial TLR2.

Disclosures: H. Lim: None. **H. Lee:** None. **K. Noh:** None. **B. You:** None. **J. Oh:** None. **H. Mok:** None. **B. Kim:** None. **S. Back:** None. **J. Park:** None. **K. Kim:** None. **R. Schnaar:** None. **M. Karin:** None. **S. Lee:** None.

Poster

616. Neuropathic Pain II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 616.06/MM5

Topic: D.02. Somatosensation: Pain

Support: JSPS KAKENHI Grant Number 26・6926

Title: Differential immune responses depending on genetic heterogeneity contribute to the phenotype of neuropathic pain among mouse strains

Authors: *K. ISAMI[1], S. IMAI[2], A. SUKEISHI[1], Y. NAKAZATO[2], K. NAGAYASU[1], H. SHIRAKAWA[1], T. NAKAGAWA[2], K. MATSUBARA[2], S. KANEKO[1];
[1]Dept. of Mol. Pharmacol., Kyoto Univ., Kyoto, Japan; [2]Dept. of Clin. Pharmacol. and Therapeut., Kyoto Univ. Hosp., Kyoto, Japan

Abstract: [Introduction] The differential neuro-immune responses due to diverse genetic factors can impact on neuronal adaptive responses associated with a phenotype of pain. However, the precise mechanisms are poorly understood. To address this issue, we compared the vulnerability of neuropathic pain in four strains of inbred mice with differential genetic backgrounds. Furthermore, we investigated how immune genetic heterogeneity can influence the pain phenotype via immune responses using these mice.
[Methods] In this study, four strains of inbred mice (C57BL/6J (B6), C3H/He (C3), DBA/2 and A/J) were used. Partial sciatic nerve ligation model was used as a neuropathic pain model, and mechanical allodynia was assessed by using von Frey filaments. Bone marrow (BM) chimeric mice were generated by intravenous injection of BM cells derived from donor mice into recipient mice irradiated with 8 Gy of gamma ray.
[Results] After the nerve injury, the most severe neuropathic pain was observed in B6, while C3 showed lower responses than other strains. In C3, the immunoreactivity of CD206-positive anti-inflammatory M2 macrophages were remarkably increased in the dorsal root ganglia (DRG) after the nerve injury, while the number of Iba1-positive activated microglia in the spinal cord was smaller than that of B6. To determine whether the phenotype of peripheral immune cells affects spinal microglial activation, we generated BM chimeric mice between B6 and C3. Behavioral and immunohistochemical studies revealed that B6 donor chimeric mice showed significant severe mechanical allodynia and decreased ratio of M2/M1 macrophages in DRG only 3 days after the nerve injury, compared to C3 donor chimeric mice, suggesting that the differential phenotype of DRG macrophages partially participated in the early phase of neuropathic pain. By contrast, differential responses of spinal microglia depended on the phenotype of recipient mice, rather than the phenotype of DRG macrophages, which mainly contributed to the development and maintenance of neuropathic pain.
[Conclusions] Taken together, our findings suggest that differential responses of immune/glial cells, especially microglia, contribute to the differential vulnerability of neuropathic pain on genetic heterogeneity.

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Poster

616. Neuropathic Pain II

Location: Halls B-H

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Program#/Poster#: 616.07/MM6

Topic: D.02. Somatosensation: Pain

Support: NIH K99DA 040016

Shirley and Stefen Hatos Center

Title: Topography of microglial activation in pain & affect related brain regions in chronic pain

Authors: *A. M. TAYLOR[1], S. MEHRABANI[1], S. LIU[2], A. TAYLOR[1], C. EVANS[1], C. CAHILL[2];
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Abstract: Microglial activation in the spinal cord plays a central role in the development and maintenance of chronic pain following a peripheral nerve injury. To date, there has not been a thorough assessment of microglial activation brain regions associated with pain and reward. To this end, we used a mouse model of neuropathic pain, whereby the left sciatic nerve was loosely constricted (chronic constriction injury) and assessed microglial activation in several brain regions two weeks following injury. We found significant upregulation of microglial markers of activation in brain regions associated with pain and affect, including the thalamus and prefrontal cortex, and amygdala. Activation was consistently most robust on the contralateral side. Regions not directly involved in pain or affective processing, such as the motor cortex, did not display microglial activation on either side. This study confirms that peripheral nerve injury induces microglial activation in regions involved with pain and affect.

Disclosures: A.M. Taylor: None. **S. Mehrabani:** None. **S. Liu:** None. **A. Taylor:** None. **C. Evans:** None. **C. Cahill:** None.

Poster

616. Neuropathic Pain II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 616.08/DP06 (Dynamic Poster)

Topic: D.02. Somatosensation: Pain

Support: P20GM103643

Title: Resident macrophages in dorsal root ganglia: phenotype, function, and neuronal surveillance

Authors: *S. A. SCARNEO, T. J. ARABATZIS, B. K. DRAGOO, R. GEGUCHADZE, D. C. MOLLIVER, K. E. HANLON;
Biomed. Sci., Univ. of New England, Biddeford, ME

Abstract: Chronic pain is a major clinical problem that has a profound effect on quality of life and poses a significant burden on the U.S. health care system. A substantial proportion of patients report inadequate management of pain and patients are often plagued by deleterious side effects. The development of persistent pain requires plasticity of primary afferent nociceptors, which drive lasting changes in central circuits. Pro-inflammatory mediators released by immune cells contribute to the sensitization of nociceptors in response to injury, suggesting that modification of inflammatory pathways may alter the perception of persistent pain. To date most attention directed towards the inflammatory component of pain has been focused on neuro-immune interactions *at the site of injury* in peripheral tissues; our recent evidence suggests macrophage activity in dorsal root ganglia (DRG) in response to distant noxious stimuli. mRNA of CCL-2, a chemokine primarily responsible for the recruitment of monocytes, is upregulated in rat DRG following peripheral injury. Changes in expression of CSF1 (M-CSF), a growth factor known to induce macrophage proliferation, has also been shown in both DRG and spinal cord following peripheral nerve injury. Further, the purinergic receptors P2Y12 and P2Y13, expressed on both macrophages and DRG neurons, have been shown to modulate anti-nociceptive effects. We have recently obtained evidence that 1) macrophages are present in mouse DRG surrounding sensory neuron cell bodies, and 2) that peripheral noxious insults (hindpaw injection of complete Freund's adjuvant or capsaicin) induce changes in macrophage phenotype and function. Our findings suggest that macrophages in DRG monitor nociceptor activity and respond to intense stimulation with functional changes. These data are critical for determining whether DRG macrophages exert positive or negative influences on nociceptor sensitization in response to peripheral insult or injury and may lead to novel therapeutic approaches for the management of persistent pain.

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Poster

616. Neuropathic Pain II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 616.09/MM7

Topic: D.02. Somatosensation: Pain

Support: ncRNA PAIN FP7 grant agreement 602133

Title: Release of exosomes containing miRNAs by macrophages in neuropathic pain

Authors: R. SIMEOLI, *M. A. MALCANGIO;
King's Col. London, London, United Kingdom

Abstract: Immune cells contribute to the mechanisms underlying neuropathic pain, at the site of nerve damage in the periphery, in the dorsal root ganglia (DRG) and in the dorsal horn of the spinal cord. At the site of injury and in the DRG, macrophages infiltrate in response to a chemokine gradient and release mediators able to sensitize neurons thereby contributing to peripheral sensitization, which together with central sensitization, is fundamental for the generation of allodynia, hyperalgesia and spontaneous pain. Here we started to explore whether macrophages release exosomes containing microRNAs (miRs) and regulate sensory neuron activity in the DRG. Exosomes are small vesicles (30-150 nm) containing RNA and protein cargos that are secreted by all cells types, including immune cells. Exosomes are considered to be highly specified enablers of intra- and extracellular communication, and recent evidence has indicated that microglia/macrophages can release and phagocytose exosomes. In our study, RT-qPCR of intracellular levels of selected miRs in cultured peritoneal macrophages demonstrated presence of miR 21-5p, Let 7b-5p, miR 155-5p and miR 134-5p. Furthermore, macrophages incubation with LPS for 3 hours resulted in significant release of exosomal markers TSG101 and CD81. Exosomes, isolated from the cell media were subjected to RT-qPCR to determine their contents. In particular, LPS stimulation induced release of miR-155, miR-21, miR-let7b, but not miR-134. Then, we evaluated whether the overexpression of miR 21-5p in macrophages could affect their phenotype. Transfection of peritoneal macrophages with miR-21-5p induced up-regulation of pro-inflammatory markers nuclear factor-κB (NF-κB) and inducible nitric oxide synthase (iNOS) and down-regulation of anti-inflammatory markers IL-10 and arginase-1. Furthermore, flow cytometry of miR 21-5p transfected macrophages confirmed in these cells a significant reduction of M2 phenotype in favor of M1. These transfection data *in vitro* indicate that an increase in intracellular miR-21-5p levels induces a shift in macrophage polarization from resting M2 phenotype to M1 pro-inflammatory phenotype. Flow cytometry analysis of leukocytes ($CD45^+$ cells) isolated from 7 day-neuropathic mice demonstrated a significant infiltration of macrophages ($F4/80^+$ $CD11b^+$ cells) in ipsilateral DRG and especially $CD206^-$ $CD11c^+$ cells (M1 macrophages) whilst $CD206^+CD11c^-$ cell (M2 macrophages) numbers were

comparable in sham and injured DRG. Future studies will assess whether the release of exosome containing miRs from macrophages contributes to the ongoing nociceptive neuron activity under neuropathic pain states.

Disclosures: R. Simeoli: None. **M.A. Malcangio:** None.

Poster

616. Neuropathic Pain II

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Topic: D.02. Somatosensation: Pain

Support: FAPESP grant 2010/12309-8

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FP7-2007-2013 HEALTH-F4-2011-281608 (TIMER)

IASP Early Career Grant Award 2011

Title: Neuro-immune activation in the sensory ganglia account for the development of herpetic neuralgia

Authors: *T. M. CUNHA[1], J. R. SILVA[2], J. TALBOT[2], A. H. LOPES[2], G. R. SOUZA[2], G. LUCAS[2], J. C. ALVES-FILHO[2], F. Q. CUNHA[2];
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Abstract: Herpetic neuralgia is the most important symptom of herpes zoster disease, which is caused by Varicella zoster. Nevertheless, the pathophysiological mechanisms involved in herpetic neuralgia are not totally elucidated. Here, we examined the neuro-immune interactions at the sensory ganglia that account for the genesis of herpetic neuralgia by using a murine model of Herpes simplex virus type-1 (HSV-1) infection. The cutaneous HSV-1 infection of mice results in the development of a zosteriform-like skin lesion followed by a time-dependent increase in pain-like responses (mechanical allodynia). Leukocytes, composed mainly of macrophages and neutrophils, infiltrate infected DRGs and account for the development of herpetic neuralgia. Infiltrating leukocytes are responsible for driving the production of TNF, which in turn mediates development of herpetic neuralgia through down-regulation of the inwardly rectifying K^+ channel, Kir4.1, in satellite glial cells. These results revealed that neuro-immune interactions at the sensory ganglia play a critical role in the genesis of herpetic

neuralgia. In conclusion, the present study elucidates novel mechanisms involved in the genesis of herpetic pain and open new avenues in its control.

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Poster

616. Neuropathic Pain II

Location: Halls B-H

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Program#/Poster#: 616.11/MM9

Topic: D.02. Somatosensation: Pain

Support: NIH/NINDS Grant R01RDE022757A

VA Merit Review Award 5I01BX000638

Title: Membrane-type 1 matrix metalloproteinase controls the release of algesic myelin basic protein epitopes and pain after constriction nerve injury

Authors: ***S. HONG**[1], A. G. REMACLE[2], J. DOLKAS[3], W. CHOI[4], S. HULLUGUNDI[3], M. ANGERT[3], T. L. YAKSH[3], T. NISHIHARA[5], A. Y. STRONGIN[2], V. I. SHUBAYEV[3]; [1]Dept. of Anesthesiol. and Pain Med., Catholic Univ. of Korea Col. of Med., Seoul, Korea, Republic of; [2]Sanford-Burnham Prebys Med. Discovery Inst., La Jolla, CA; [3]Dept. of Anesthesiol., UCSD, La Jolla, CA; [4]Sungkyunkwan Univ. Col. of Med., Seoul, Korea, Republic of; [5]Ehime Univ. Col. of Med., Toon, Japan

Abstract: Myelin basic protein (MBP) is an auto-antigen we have implicated in the development of severe pain arising from innocuous touch (mechanical allodynia). The specific mechanisms of this pro-algesic MBP activity remain obscure. Herein, we have demonstrated that following chronic constriction injury (CCI) to rat sciatic nerve, membrane-type (MT)1-matrix metalloproteinase (MT1-MMP/MMP-14) contributes to the release of the pro-algesic MBP69-86 peptide and then to mechanical allodynia. Specifically, MT1-MMP was constitutively expressed in the intact sciatic nerve. However, there was a gradual MT1-MMP increase starting at day 3 post-CCI, especially in Schwann cells and macrophages. Inhibition of MT1-MMP activity, using the selective, function-blocking human DX2400 monoclonal antibody, administered once into the sciatic nerve at day 3 post-CCI, attenuated mechanical allodynia. Morphologically, CCI nerves displayed Wallerian degeneration (axonal degeneration, edema, myelin ovoids and immune cell infiltration) after IgG1 treatment, whereas greater numbers of uncompromised axons were observed in CCI nerves treated with DX2400. DX2400 treatment also prevented

MBP degradation and reduced the level of the liberated MBP69-86 peptide at the CCI site. In teased out sciatic nerve myelinated fibers, we observed that after nerve injury, a decline in intact MBP was accompanied by accumulation of the membrane MT1-MMP and the MBP69-86-reactive fragment in close proximity to the nodes of Ranvier. Finally, we confirmed our earlier *in vitro* findings that MT1-MMP liberated the 69-86 MBP fragments from the intact MBP, and *in vivo* observations that the MBP69-86 peptide (rat sequence) injection into the intact nerve was sufficient to produce a robust and lasting state of allodynia. These data implicate MT1-MMP as a key protease in sustaining MBP-induced pain and an important therapeutic target in painful neuropathy.

Disclosures: **S. Hong:** None. **A.G. Remacle:** None. **J. Dolkas:** None. **W. Choi:** None. **S. Hullugundi:** None. **M. Angert:** None. **T.L. Yaksh:** None. **T. Nishihara:** None. **A.Y. Strongin:** None. **V.I. Shubayev:** None.

Poster

616. Neuropathic Pain II

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Topic: D.02. Somatosensation: Pain

Support: Queen Elizabeth II Graduate Scholarship

Faculty of Medicine & Dentistry/Alberta Health Services Graduate Student Recruitment Scholarship

Multiple Sclerosis Society of Canada Studentship

Multiple Sclerosis Society of Canada Operating Grant

Title: Facial pain hypersensitivity and trigeminal pathology in an animal model of CNS inflammation and demyelination

Authors: *K. C. THORBURN[1], J. W. PAYLOR[2], C. A. WEBBER[3], I. R. WINSHIP[2], B. J. KERR[4];
[1]Pharmacol., [2]Psychiatry, [3]Anat., [4]Anesthesiol. and Pain Med., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Trigeminal neuropathic pain is a well-recognized complication of the demyelinating disease multiple sclerosis (MS). The mechanisms underlying MS-related trigeminal neuropathic pain are poorly understood. This can be attributed, at least in part, to the lack of an animal model

that exhibits trigeminal pathology similar to that described in MS. Experimental autoimmune encephalomyelitis (EAE) is an animal model characterized by central nervous system inflammation and demyelination that is commonly used to study the pathophysiology of MS. We show here that mice with EAE exhibit increased sensitivity to air puffs applied to the whisker pad. The increased sensitivity to air puff stimulation is accompanied by immune cell infiltration and glial activation at several points along the trigeminal primary afferent pathway. Specifically, we found increased numbers of T cells and macrophages in the trigeminal ganglia (TG) of mice with EAE. We also observed a significant, but transient, increase in satellite glial cell expression of GFAP in the TG of EAE animals. Further investigation of the TG in EAE animals has revealed that the neuronal injury marker, ATF3, is also transiently increased. Additionally, recent preliminary data suggest that there are more calcitonin gene-related peptide (CGRP)-positive neurons in the TG of EAE animals. At the level of the trigeminal root entry zone, we found significant numbers of immune cells, demyelination and a transient decrease in GFAP immunoreactivity in EAE mice. Finally, we observed T cell infiltration, microglia/macrophage activation, demyelination and reactive astrocytes throughout the trigeminal brainstem complex in EAE animals. Interestingly, the pattern of trigeminal demyelination seen in EAE mice is similar to that previously described in people with MS-related trigeminal neuropathic pain. This is the first study to show orofacial sensory disturbances and trigeminal demyelination in EAE. Collectively, our data suggest that EAE may be a useful model for understanding MS-related trigeminal neuropathic pain conditions such as trigeminal neuralgia.

Disclosures: K.C. Thorburn: None. **J.W. Paylor:** None. **C.A. Webber:** None. **I.R. Winship:** None. **B.J. Kerr:** None.

Poster

616. Neuropathic Pain II

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Topic: D.02. Somatosensation: Pain

Support: NIH NS064341

DE021847

Title: Mechanism of thrombospondin-4 and alpha2delta-1 dependent processes in modulating excitatory synaptogenesis underlie neuropathic pain processing in nerve injury models

Authors: *Y. P. YU[1], B. VO[1], D. Z. LUO[2];
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Abstract: We have shown previously that spinal nerve ligation-induced peripheral nerve injury leads to increased production of extracellular matrix glycoprotein Thrombospondin-4 (TSP4) and of voltage gated calcium channel (VGCC) accessory subunit α2δ-1. Furthermore, interdependent interactions of TSP4 and α2δ-1 result in excitatory synaptogenesis that correlates with behavioral hypersensitivity. However, very little is known about the mechanism underlying TSP4/α2δ-1 mediated synaptogenesis. We examined this issue using a primary dorsal root ganglion (DRG)/spinal cord neuron co-culture system as an *in vitro* model that mimics the connectivity between primary DRG sensory neurons and secondary spinal cord neurons. We have found that TSP4 interacts specifically with pre-synaptic α2δ-1 at the central terminals of DRG neurons to promote synaptogenesis, but TSP4 interactions with post-synaptic α2δ-1 at spinal cord neurons or α2δ-1 at DRG neuronal somas are not synaptogenic. Early gabapentin treatments can block initiation of excitatory synaptogenesis mediated by TSP4/α2δ-1 dependent processes, but delayed gabapentin treatments have no effect on already formed excitatory synapses. Furthermore, excitatory synaptogenesis mediated by TSP4/α2δ-1 dependent processes is T-type VGCC function dependent as it can be blocked by T-type VGCC specific blockers, but not by N-type, L-type and P/Q-type VGCC blockers. Together, these findings support that nerve injury-induced excitatory synaptogenesis in dorsal spinal cord is likely initiated by aberrant TSP4 interactions with pre-synaptic α2δ-1 that activate a synaptogenic pathway involving T-type VGCC functions. Blocking this pathway preemptively may provide beneficial effects in preventing injury-induced aberrant excitatory synaptogenesis and chronic pain states.

Disclosures: **Y.P. Yu:** None. **B. Vo:** None. **D.Z. Luo:** None.

Poster

616. Neuropathic Pain II

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Program#/Poster#: 616.14/MM12

Topic: D.02. Somatosensation: Pain

Title: Chronic constriction injury of the sciatic nerve results in limited changes of polymodal spinal convergent neurons responses in the anesthetized rat

Authors: *J. ALLARD[1], C. LE CUDENNEC[2], V. CASTAGNÉ[2];
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Abstract: Chronic constriction injury (CCI) following loose ligation of the sciatic nerve induces robust behavioral mechanical and thermal hypersensitivity in rats. The aim of the present work was to assess whether “basic” electrophysiological recordings of spinal neurons in CCI and SHAM-operated rats, including lamina I nociceptive specific (NS) and lamina V wide dynamic

range (WDR) projection neurons, reflected this expected hyper-reactivity. Spinal neurons innervating the glabrous skin of the hind paw were recorded under pentobarbital or isoflurane anaesthesia in 2 experiments including 10 CCI and SHAM rats each. Spontaneous activity, evoked and post-discharge responses to mechanical (von Frey probe, calibrated pinch) and thermal (Water Jet) stimuli were measured on the side ipsilateral and contralateral to the surgery. The experiments performed under pentobarbital or isoflurane anaesthesia focussed on WDR and NS neurons, respectively. A total of 166 neurons were recorded, yielding approximately 20 neurons for each combination of side, pathophysiological and anaesthetic condition. Neurons recorded under pentobarbital included mostly WDR profiles with receptive field covering the digits. Neurons recorded under isoflurane included mostly NS profiles with receptive field covering the palm of the paw, half of them being confirmed projection neurons by antidromic stimulation from the cervical cord. In the pentobarbital experiment, evoked responses and to a greater extent post-discharge responses were slightly increased on the injured side in CCI rat compared to the treated side in SHAM rats. Comparing the side ipsilateral to the surgery with the contralateral side showed that on the ipsilateral side responses altogether tended to be *increased* in CCI rats, but *decreased* in SHAM rats. In the isoflurane experiment, responses altogether were almost similar on the treated side in CCI and SHAM rats. Evoked responses were similar when comparing the ipsilateral to the contralateral side in CCI and SHAM rats. In contrast, post-discharge responses were greatly increased on the ipsilateral side to the injury in CCI rats compared to the contralateral side. No difference was observed in SHAM rats.The present experimental conditions did not provide evidence for the expected sensitization of spinal neurons in CCI rats. We suspect that more stringent criteria of inclusions are necessary to unravel the spinal neural substrate at the origin of behavioral hypersensitivity. Our data also suggest that the choice of appropriate control conditions is needed to interpret the effects of CCI on the activity of spinal neurons.

Disclosures: J. Allard: None. **C. Le Cudennec:** None. **V. Castagné:** None.

Poster

616. Neuropathic Pain II

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Program#/Poster#: 616.15/NN1

Topic: D.02. Somatosensation: Pain

Support: NIH/NINDS, R01RDE022757A

VA Merit Review Award, 5I01BX000638

Title: Myelin basic protein auto-antigen in pain from light touch

Authors: *S. K. HULLUGUNDI[1,2], A. V. CHERNOV[3], A. G. REMACLE[3], K. A. EDDINGER[1], M. ANGERT[1,2], J. DOLKAS[1,2], J. B. LEUNG[1], A. Y. STRONGIN[3], T. L. YAKSH[1], V. I. SHUBAYEV[1,2];
[1]Dept. of Anesthesiol., Univ. of California San Diego, LA Jolla, CA; [2]VA San Diego Healthcare Syst., La Jolla, CA; [3]Sanford-Burnham-Prebys Med. Discovery Inst., La Jolla, CA

Abstract: Myelin basic protein (MBP) is a known auto-antigen detected in multiple sclerosis patients. Our previous studies have shown that immunodominant MBP epitopes are proteolytically released in sciatic nerves after chronic construction injury (CCI). A single injection into the sciatic intact nerve of the pure peptides that represented these MBP epitopes was sufficient to produce a sustained state of neuropathic pain. The MBP peptides produced mechanical, but not thermal pain hypersensitivity, as determined using von Frey and Hargreaves testing. These data imply the role of myelinated A-afferents, which are conventionally known to transduce touch stimuli, in pain transduction. The present study is focused on understanding the mechanisms underlying the activities of the pro-algesic MBP peptides in Schwann cell and myelinating DRG cultures. The ability of the endogenous and exogenous pro-algesic MBP peptides to undergo axonal transport is also being assessed. Finally, mechanical hypersensitivity induced by MBP was transiently inhibited with gabapentin (a ligand of voltage-gated calcium channel α2δ1), an interleukin (IL)-6-neutralizing antibody, and was diminished in T-cell deficient athymic nude rats. In contrast, therapeutics such as lidocaine (sodium channel blocker), ketorolac (COX inhibitor), MK801 (NMDA antagonist) and TAK242 (toll-like receptor (TLR)-4 inhibitor) failed to reverse MBP-induced pain. Together, our data predominantly implicate adaptive T cells but not innate immune cells, such as microglia, in mediating MBP-induced pain. Because our initial studies have been accomplished in females, we now focus on the comparative analyses of the effects of nociceptive MBP peptides in female vs. male animals. In summary, MBP presents a novel pharmacological target in the nociceptive cascades activated in demyelinating disorders and painful neuropathies.

Disclosures: S.K. Hullugundi: None. **A.V. Chernov:** None. **A.G. Remacle:** None. **K.A. Eddinger:** None. **M. Angert:** None. **J. Dolkas:** None. **J.B. Leung:** None. **A.Y. Strongin:** None. **T.L. Yaksh:** None. **V.I. Shubayev:** None.

Poster

616. Neuropathic Pain II

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Topic: D.02. Somatosensation: Pain

Support: Department of Anesthesiology Startup Funds (TV)

Title: 5-hydroxymethylcytosine and ten-eleven translocation proteins in the dorsal root ganglia—expression and dynamic regulation in neuropathic pain

Authors: *M. CUMMINS[1], A. CHAMESSIAN[2], M. QADRI[1], M. HENDRICKSON[1], T. BUCHHEIT[1], T. BERTA[3], T. VAN DE VEN[1];
[1]Anesthesiol., [2]Duke Univ., Durham, NC; [3]Anesthesiol., Univ. of Cincinnati, Cincinnati, OH

Abstract: Epigenetic mechanisms are increasingly recognized to contribute to the pathology of chronic pain conditions. 5-hydroxymethylcytosine (5hmC) is a recently discovered epigenetic modification with wide-ranging roles in neuronal processes such as development, learning and memory and synaptic transmission. Although much is known about 5hmC in the central nervous system, it is not yet known whether this epigenetic mark is present in the somatosensory neurons of the dorsal root ganglia (DRG) or if it plays a role in the pathogenesis of neuropathic pain due to nerve injury. Thus, the aim of this current study was characterize the expression and nerve injury-induced dynamics of 5hmC and the 5hmC-generating Ten-eleven translocation (TET1-3) proteins in the DRG. We found that 5hmC is present in both neurons and glia of the DRG and that 5hmC levels increase in the DRG after peripheral nerve injury. In addition, all three TET family members were found to be expressed in DRG cells, but with distinct expression patterns. TET1 and TET3 demonstrated exclusively neuronal expression, while TET2 was found broadly in neurons and non-neuronal cells. Interestingly, TET3 was preferentially expressed in small and medium diameter, Peripherin-positive DRG neurons and was the only TET family member whose expression was altered by peripheral nerve injury, indicating that TET3 may play a distinct role in nociception under normal and pathological conditions. Taken together, our study suggests that 5hmC and TET proteins may be important contributors to the pathogenesis of neuropathic pain and warrant further investigation. To test the specific contributions of TET1-3 in neuropathic pain, we are currently conducting studies using shRNA-mediated knockdown of TET1-3 *in vivo*.

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Poster

616. Neuropathic Pain II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 616.17/NN3

Topic: D.02. Somatosensation: Pain

Support: Mission Connect (TIRR Foundation)

Title: Autonomic dysfunction contributes to nociceptive plasticity after spinal cord injury

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Abstract: Chronic neuropathic pain is a major consequence of spinal cord injury (SCI). As a result of its unpredictable onset, various etiologies and expressions, the underlying neural mechanisms of chronic neuropathic pain are poorly understood. SCI also often results in autonomic dysfunction which typically includes compromised respiratory function. There is evidence in support of significant cellular and anatomical overlaps between maladaptive sympathetic and sensory processing. However, a thorough investigation into their interactions after SCI has not been demonstrated.

The objective of the study was to investigate temporal changes in sensory nociceptive processing and autonomic dysfunction after SCI in adult Long Evans rats with a lateral hemisection or contusion SCI, or sham surgery at the thoracic (T) 8 spinal cord. Specifically, we focused on the development of thermal and mechanical hypersensitivity and parallel changes in respiratory rates (RRs). At weekly time-points ranging from 24 hours to 7 weeks after injury, thermal sensitivity was assessed with the tail-flick and Hargreaves tests, while mechanical responses to von Frey filaments and brush stimulation to the trunk skin were evaluated. Changes in RRs were assessed using highly sensitive non-contact electric field sensors (EPIC, Plessey Semiconductors) that measure breathing frequencies. RRs were monitored before and after SCI, and during behavioral pain tests.

Our results showed that SCI increases basal respiratory rates, an increase that is maintained for several weeks after injury. SCI rats had increased thermal and mechanical sensitivity. Moreover, they showed a greater increase in their RRs in response to mechanical and thermal stimulation, although this effect depended on the type of injury. In SCI rats with a hemisection, RRs were increased during thermal testing. In contrast, an increase in RRs in response to mechanical testing was typically seen in subjects with a moderate contusion injury. Mechanical allodynia and stimulation-induced increases in RRs were in part driven by increased sympathetic activity, as these effects were significantly attenuated following administration of propranolol, a β-adrenergic antagonist (20mg/kg).

Overall, these preliminary observations reveal that maladaptive sensory processing after SCI is influenced by autonomic dysfunction. They also suggest that interactions between sensory and autonomic dysfunction after SCI may depend on the type or severity of injury.

Disclosures: S.M. Garraway: None. **S. Parvin:** None.

Poster

616. Neuropathic Pain II

Location: Halls B-H

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Ivy A Thomson and William A Thomson

Title: Changes in chloride co-transporter and cytoskeletal proteins in mice with EAE: Implications for pain processing in Multiple Sclerosis.

Authors: *M. YOUSUF[1], K. ZUBKOW[1], B. J. KERR[2];
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Abstract: Multiple Sclerosis (MS) is an autoimmune inflammatory disorder of the central nervous system. It manifests with a multitude of physical and cognitive symptoms. More than half of MS patients also complain of neuropathic pain. Neuropathic pain can arise as a result of hyperexcitability of spinal dorsal horn neurons. Various proteins are important for regulating the balance between excitation and inhibition in the CNS to prevent hyperexcitability. The Na^+-K^+-Cl^- co-transporter 1 (NKCC1) is one such transporter that is crucial for determining the strength and polarity of GABA, the principle inhibitory neurotransmitter in the CNS. NKCC1 on presynaptic terminals in the dorsal horn regulates primary afferent depolarization. We set out to identify whether changes in the expression of NKCC1 arises in mice with experimental autoimmune encephalomyelitis (EAE), an animal model commonly used to study MS, that may relate to pain hypersensitivity in this model. qRT-PCR analysis of dorsal spinal cord revealed a marked decrease in mRNA levels for NKCC1 at disease onset and peak, time points when pain behaviours are prominent in the disease. A similar pattern of expression was found at the protein level for both NKCC1 and phosphorylated NKCC1, in the dorsal spinal cord. In contrast, we find that in the DRG, protein levels of NKCC1 are significantly elevated at the onset of clinical signs in EAE. An accumulation of NKCC1 in the DRG with a reduction in the dorsal horn may be the result of impaired anterograde transport from the cell bodies of DRG neurons. We find that the

axon-specific microtubule associated protein, tau, is downregulated after the onset of symptoms. Furthermore, kinesin levels are also decreased at the onset of clinical signs in EAE. These results suggest that the axon cytoskeleton is integral for mediating the levels of the chloride co-transporter NKCC1 in the axon terminals of the dorsal horn. Early disease associated damage to peripheral cytoskeleton proteins may prevent NKCC1 transport leading to a reduction in presynaptic inhibition, hyperexcitability and enhanced nociceptive behaviours early in the disease course of EAE.

Disclosures: **M. Yousuf:** None. **K. Zubkow:** None. **B.J. Kerr:** None.

Poster

616. Neuropathic Pain II

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Support: NIH R03DA26734

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Title: SIRT3 and mitochondrial ROS play an important role in HIV neuropathic pain induced by the antiretroviral therapy in rats

Authors: S. LIU, T. IIDA, H. YI, K. TAKAHASHI, D. LUBARSKY, *S. HAO;
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Abstract: Highly active antiretroviral therapy (HAART) has markedly increased survival of patients with HIV/AIDS. However, nucleoside reverse transcriptase inhibitors (NRTIs) induce neurotoxicity and cause a painful peripheral neuropathy. While there has been a major shift away from some neurotoxic NRTIs in current antiretroviral therapy, a large number of HIV patients alive today have previously received them, and many have developed painful peripheral neuropathy. The molecular mechanisms by which NRTIs contribute to the development of neuropathic pain, are not known. Mitochondrial oxidative stress is involved in the neuroinflammatory diseases. Sirtuin-3 (SIRT3) is a class III lysine deacetylase that is localized to the mitochondria, and regulates mitochondrial respiration and oxidative stress resistance enzymes, such as manganese superoxide dismutase (MnSOD). SIRT3 deacetylates MnSOD and

activates MnSOD to scavenge ROS (mitochondrial anti-oxidative mechanisms (SIRT3-MnSOD)). Few studies show the effect of SIRT3-MnSOD on HIV neuropathic pain. Here, we examined if SIRT3 and mitochondrial oxidative stress played a role in HIV neuropathic pain. Neuropathic pain was induced by systemic 2',3'-dideoxyinosine (ddI, one of NRTIs). The lowered mechanical threshold (mechanical allodynia) induced by ddI, lasted for 5 weeks. Systemic ddI induced the downregulation of SIRT3, and decreased the activity of MnSOD in the spinal cord dorsal horn. Intrathecal injection of Mito-tempol (MitoT, a mitochondria-targeted superoxide scavenger), or recombinant SIRT3 increased mechanical threshold. Intrathecal Mito-T suppressed MitoSox (mitochondrial superoxide marker) profile neuron number in the spinal cord dorsal horn. Based on these results above, we suggest that SIRT3 and mitochondrial oxidative stress play an important role in HIV neuropathic pain induced by NRTIs. The study will provide preclinical evidence for novel pharmacotherapy in patients with HIV-related pain.

Disclosures: **S. Liu:** None. **T. Iida:** None. **H. Yi:** None. **K. Takahashi:** None. **D. Lubarsky:** None. **S. Hao:** None.

Poster

616. Neuropathic Pain II

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant R01 NS031680

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Title: Mitochondrial reactive oxygen species facilitate excitatory synapses on spinothalamic tract neurons in neuropathic pain

Authors: ***C. BAE**, J. WANG, A. BITTAR, H. SHIM, J.-H. LA, S.-J. TANG, J. CHUNG; Dept. of Neurosci. and Cell Biol., Univ. of Texas Med. Br. At Galveston, Galveston, TX

Abstract: Synaptic plasticity is important for normal brain functions such as learning and memory. Dysregulated synaptic plasticity is implicated in the development of various neurological conditions including neuropathic pain. However, the underlying mechanism of synaptic plasticity in neuropathic pain conditions is poorly understood. We previously reported that the level of mitochondrial reactive oxygen species (mROS) is correlated with the extent of allodynia induced by intradermal capsaicin. This indicates that the sensitization of the spinal

dorsal horn neurons is affected by mROS. The present study explored the possible involvement of mROS in the plasticity of excitatory synapses on the spinal dorsal horn neurons in neuropathic condition by using 3 types of mice: mitochondrial superoxide dismutase (SOD2) knock-out (SOD2-KO), SOD2-overexpressing (SOD2-OE) transgenic, and wild type (WT). Neuropathic mice were generated by spinal nerve ligation (SNL) surgery. Pain levels were determined by measuring the foot withdrawal frequency to von Frey filament stimulation before and after SNL up to 14 days. Miniature excitatory postsynaptic currents (mEPSC) in identified spinothalamic tract neurons (STTn) were recorded from the spinal cord slices using patch clamping. Patch clamp recordings were done 7-14 days after SNL. SOD2-KO showed greater but SOD2-OE manifested less mechanical allodynia than WT mice after SNL. Comparable with the pain behavior, SNL increased the frequency of mEPSC in STTn in the order of SOD2-KO > WT > SOD2-OE. The amplitude of mEPSC did not differ either among three genotypes or between sham- and SNL-operated mice. Thus, we conclude that mROS are important molecules increasing activities of excitatory synapses on STTn, thereby contributing to neuropathic mechanical allodynia.

Disclosures: **C. Bae:** None. **J. Wang:** None. **A. Bittar:** None. **H. Shim:** None. **J. La:** None. **S. Tang:** None. **J. Chung:** None.

Poster

616. Neuropathic Pain II

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Topic: D.02. Somatosensation: Pain

Support: Department of Defense DM102108

Title: A single intrathecal delivery of the adenosine 2A receptor agonist, ATL313, produces sustained antinociception by promoting A2BR- and PPAR-gamma-mediated anti-inflammatory processes in the spinal cord of rats.

Authors: ***A. J. KWILASZ**[1], S. M. GREEN FULGHAM[1], H. P. PATEL[1], L. CHANTE DURAN-MALLE[1], S. TILDEN[1], J. RIEGER[2], S. F. MAIER[1], L. R. WATKINS[1]; [1]Psychology and Neurosci., Univ. of Colorado-Boulder, Boulder, CO; [2]Adenosine Therapeut., Charlottesville, VA

Abstract: Neuropathic pain affects approximately 4 million people in the U.S. and remains refractory to analgesics in many patients. We have previously found that adenosine 2A receptor (A2AR) agonists such as ATL313 generate profound anti-inflammatory effects and produce

sustained reversal of mechanical allodynia when administered intrathecally (IT) in rat models of neuropathic pain, including chronic-constriction-injury (CCI). In this study, we further explore spinal cord mechanisms related to the sustained antinociceptive effects of ATL313. Male Sprague Dawley rats received CCI surgery which produces long-lasting mechanical allodynia for several months. On day 14 following CCI, a single IT administration of ATL313 effectively reversed mechanical allodynia for four weeks. IT delivery of an A2BR antagonist, MRS1754 but not an A2AR antagonist, ZM241385, one week after ATL313 administration, permanently reversed the sustained antinociceptive effect. This effect was correlated with increased spinal cord A2BR mRNA expression in ATL313-treated animals that received CCI but not sham surgery. Moreover, IT delivery of the peroxisome-proliferator-activated receptor gamma (PPAR-γ) antagonist, GW9662, temporarily reversed the sustained antinociceptive effect produced by ATL313. Isolation of spinal cord cells from animals treated with ATL313 *in vivo* revealed that these cells release IL-10 and cyclic adenosine monophosphate (cAMP). These results suggest that IT A2AR agonists produce sustained reversal of neuropathic pain by promoting A2BR- and PPAR-γ-related anti-inflammatory signaling in spinal cord, associated with release of cAMP and IL-10.

Disclosures: **A.J. Kwilasz:** None. **S.M. Green Fulgham:** None. **H.P. Patel:** None. **L. Chante Duran-Malle:** None. **S. Tilden:** None. **J. Rieger:** None. **S.F. Maier:** None. **L.R. Watkins:** None.

Poster

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Title: Time course characterization of bone loss following K/BxN serum transfer in male and female mice

Authors: S. WOLLER[1], A. MARTINEZ-MARTINEZ[2], M. CORR[1], T. L. YAKSH[1], *J. JIMENEZ-ANDRADE[2];
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Abstract: Joint pain is the primary reason individuals with arthritis seek medical care. Unfortunately, arthritic pain is difficult to treat, and current treatments are fraught with undesirable side effects. It is commonly thought that pain is evoked by inflammation and / or joint damage, leading to a peripheral and central sensitization. However, the severity of pain often does not correlate well with these factors. We hypothesize that, while inflammation leads to sensitization of joint terminals, over time, changes occurring within the joint itself (e.g. bone loss / remodeling) may contribute to the ongoing pain state. Therefore, our goal was to characterize the time-course of changes occurring within cortical and trabecular bone of the femur, tibia, and ankle joint of arthritic mice using μCT analysis.
Using the K/BxN serum transfer model of arthritis, we have shown that male C57Bl/6 mice develop clinical signs of arthritis peaking around 12 days after serum transfer and declining thereafter. Inflammation is accompanied by a robust tactile allodynia (TA) commensurate with the onset of inflammation. Importantly male, but not female mice show TA persisting well beyond the resolution of inflammation (day 28). For this reason, bones of arthritic male and female mice were analyzed on day 0 (naïve), day 10, and day 28.
We quantified arthritis-induced changes in the femur, tibia, talus, and calcaneus. For trabecular bone, we determined trabecular bone mineral density (tBMD), percent bone volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), the degree of anisotropy (DA); while for cortical bone, BMD (cBMD), cortical area (Ct.Ar), and cortical thickness (Ct.Th) were examined.
In the distal femur and proximal tibia, we found that arthritic male, but not female, mice showed a significant decrease in tBMD, BV/TV, Tb.N at day 28 as compared to age- matched naive mice. There were no major changes in the cortical bone of the femoral or tibial mid-diaphyses. In the talus, there were no significant changes in any trabecular bone parameters in female or male arthritic mice as compared to naïve. In the calcaneus, tBMD and BV/TV are significantly decreased in male and female arthritic animals as compared to naïve. While there was no difference in Tb.Th or Tb.Sp, we found a significant decrease in Tb.N over time in arthritic females.
Together, these results suggest that K/BxN serum transfer-induced arthritis results in sex-dependent changes in TA and bone loss in different bones. Additional work will determine whether these bone changes are associated with long-term mechanical hypersensitivity in arthritic mice.

Disclosures: S. Woller: None. **A. Martinez-Martinez:** None. **M. Corr:** None. **T.L. Yaksh:** None. **J. Jimenez-Andrade:** None.

Poster

616. Neuropathic Pain II

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant 1R03NS096454-01

Title: Alterations in afferent pathway signaling and neurogenic inflammation following spinal cord injury

Authors: *J. R. YASKO[1], E. E. YOUNG[2], K. M. BAUMBAUER[2];
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Abstract: Spinal cord injury (SCI) can lead to a devastating loss of function and intractable chronic pain.Pain is consistently ranked as one of the most significant health concerns among patients, and many report receiving inadequate pain relief. Pain following SCI is unique because it is one of few instances where pain is generated centrally. However, evidence has supported that the cells within the spinal cord are not the only cells in the nociceptive system impacted by SCI and that primary afferent nociceptors also play an integral role in the development and maintenance of chronic SCI-induced pain. Studies examining the contribution of primary afferents to SCI pain have shown that sensory neurons exhibit spontaneous activity following SCI. The neurons that exhibit spontaneous activity are sensitive to capsaicin, possess the TRPV1 receptor, and their spontaneous activity can be reduced by silencing the nociceptor specific sodium channel, Nav1.8. Sensitization of the primary afferent nociceptors can also contribute to the development of neurogenic inflammation whereby proinflammatory molecules are released from the peripheral terminals of nociceptors, resulting in peripheral inflammation and enhancement of pain. Here we examine how SCI impacts sensory neurons, as well as the skin and muscle in which they innervate. Mice were given a severe SCI using compression clips (60 g of force) at the level of T10-11, producing complete paralysis below the level of the forelimbs. To determine the time course of alterations in targets of interest, mice were sacrificed 24 hr following SCI and skin, muscle, and dorsal root ganglia (DRG) were collected at, above, and below the level of SCI. Tissue was then processed and analyzed for changes in gene expression using real-time RT-PCR. Analysis of DRG tissue revealed significant increases in TRPV1, TRPA1, calcα (CGRP), TrkA, and P2X3. Similar increases in gene expression were observed in DRGs collected from all levels with respect to the site of injury, for the exception of TRPV1, which showed significantly greater increases in expression above the level of injury. Analysis of hind and forepaw muscle showed significant increases in the expression of TRPV1 and calcα in hindpaw muscle, TRPA1 in forepaw muscle, and TrkA in hind- and forepaw muscle. Ongoing experiments are examining the time course of additional changes in gene and protein expression,

alterations within individual afferents, and how severity of injury impacts these changes. Our data suggest that SCI results in early sensitization of afferent pathways and the development of neurogenic inflammation in a temporally specific manner.

Disclosures: J.R. Yasko: None. **E.E. Young:** None. **K.M. Baumbauer:** None.

Poster

616. Neuropathic Pain II

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Topic: D.02. Somatosensation: Pain

Support: FAPDF

DPP-UnB

Title: Antinociceptive effect of a modified protonectin isolated from Parachartergus fraternus wasp

Authors: *P. GALANTE[1], J. GONÇALVES[2], E. F. SCHWARTZ[2], L. P. SILVA[2], M. MORTARI[2];
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Abstract: Neuroactive compounds are of great interest for their relevant potential to design new drugs, important to prevent and/or treat diseases such as chronic pain, as well as to develop other pharmacological tools. In this context, the study of wasp venoms has lead to the discovery of neuroactive molecules, however most peptides have yet to be identified or characterized. The social wasp *Parachartergus fraternus* has a remarkable peculiarity: although these wasps live in community, their venom act by irreversibly and non-lethally paralyzing their prey, which differs from other social wasps, which typically uses the venom only for protection against prey. This difference in the mode of action suggests the presence of compounds with activity in the nervous system. For this reason, the aim of this study was the identification and isolation of antinociceptive peptides in the venom of *P. fraternus.* The venom of *P. fraternus* was analyzed by two complementary strategies: HPLC fractionation followed by MALDI-TOF analysis and cDNA library construction and sequencing. A specific fraction was identified by mass spectrometry, however sequencing of this fraction was unable to unambiguously determine between Leucine and Isoleucine. Using the transcriptomic approach, the sequence of the precursor encoding this peptide was identified. The precursor encodes a propeptide of 61 amino acids with a putative signal peptide. The sequence of the mature peptide was determined and a

synthetic peptide with two amino acid substitutions was produced by solid phase synthesis. Antinociceptive activity of both peptides was evaluated by the hot plate model in Swiss mice (n=4-6/group). The peptides were infused via i.c.v. at doses of 16 and 8 nmol/animal four days after the implantation of the guide cannula. Morphine (16nmol/animal) was used as positive control and vehicle solution was used as negative control (2μL/animal). The analgesic activity of the natural isolated peptide was shown to be effective at 8 nmol/animal ($p<0.05$) in the time points of 90, 120 and 240 min whereas the higher dose of 16 nmol/animal showed a decreased effect, probably due to neurotoxicity. The synthetic peptide had a dose-dependent response, where the highest dose (16 nmol/animal) showed antinociceptive activity that did not differ ($p<0.05$) from morphine in all time points tested. The synthetic peptide did not show hemolytic activity. Animals injected with the higher dose of the synthetic peptide did not show motor deficit in the antinociceptive test. This synthetic peptide tested showed the potential to contribute to the development of neuroscience in the elucidation of synaptic transmission and in the design of new drugs.

Disclosures: P. Galante: None. **J. Gonçalves:** None. **E.F. Schwartz:** None. **L.P. Silva:** None. **M. Mortari:** None.

Poster

616. Neuropathic Pain II

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant NS065926

Title: Methylglyoxal produces endoplasmic reticulum stress response in drg neurons

Authors: *P. BARRAGAN-IGLESIAS, T. J. PRICE;
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Abstract: The endogenous reactive metabolite methylglyoxal (MGO) has been suggested to play a causal role in the pathogenesis of diabetic peripheral neuropathic pain. Evidences suggest that MGO activates TRPA1 and $Na_{V1.8}$ channels in sensory neurons to produce pain. However, these events may occur only at very high concentrations and the mechanisms underlying MGO-induced pain over the longer term are not known. Recently the endoplasmic reticulum (ER) stress pathway was proposed to regulate the excitability of the nociceptive system, potentially via a direct action in DRG neurons. We hypothesized that MGO induces ER stress in DRG leading to a sensitization of these neurons. Our preliminary results using primary DRG cultures and WB

show that MGO (1 micromolar, 3-48 h) produces an up-regulation of ER stress markers BiP, p-PERK and p-eIF2 alpha. Remarkably, chronic ER stress response was observed with long exposure to MGO (24-48 h). Moreover, the integrated stress response inhibitor ISRIB (200 nanomolar, 24 h) attenuated the effects of eIF2α phosphorylation produced by MGO exposure. Experiments *in vivo* and *in vitro* are ongoing in our lab to determine the mechanisms and downstream targets associated with MGO-ER stress response to promote chronic pain.

Disclosures: P. Barragan-Iglesias: None. **T.J. Price:** None.

Poster

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Topic: D.02. Somatosensation: Pain

Support: NINDS NS082746

NIDCR DE025551

Title: AKAP-dependent modulation of GRK2 maintains delta opioid receptor incompetence

Authors: *A. D. BRACKLEY[1,2], R. GOMEZ[3], K. A. GUERRERO[3], N. A. JESKE[2,3,4]; [2]Pharmacol., [3]Oral & Maxillofacial Surgery, [4]Physiol., [1]UTHSCSA, San Antonio, TX

Abstract: We recently identified a novel, non-internalizing role for G protein-coupled receptor kinase 2 (GRK2) in maintaining delta opioid receptor (DOR) analgesic incompetence at the plasma membrane. However, the mechanism that maintains constitutive GRK2 association with DOR remains to be elucidated. Previous studies have shown that PKA phosphorylation of GRK2 plays an important role in its plasma membrane targeting. A-kinase anchoring protein 79/150 (AKAP), a scaffolding protein expressed in sensory neurons that resides at the plasma membrane, scaffolds protein kinase A (PKA) and mediates downstream signal transduction. In the present study, we sought to determine whether constitutive DOR desensitization by GRK2 is directed by PKA via AKAP-scaffolding. siRNA-mediated knockdown of AKAP in primary neuronal cultures significantly increased basal DOR association with GRK2. Complementary studies in primary sensory neurons from AKAP wildtype mice demonstrated that DOR can be primed for activation, but not in AKAP (-/-) neurons. In overexpression studies with wildtype and mutant AKAP, only AKAPΔPKA significantly reduced plasma membrane DOR:GRK2 association and consequently increased DOR activity in sensory neurons without the need for priming. Furthermore, AKAP expression augments GRK2 phosphorylation by PKA in the

cytosol and plasma membrane targeting of GRK2. These findings suggest that AKAP scaffolds PKA to increase plasma membrane targeting of GRK2 and maintain DOR analgesic incompetence.
Research supported by NINDS NS082746 (NAJ) and NIDCR DE025551 (ADB).

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Poster

616. Neuropathic Pain II

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Topic: D.01. Sensory Disorders

Support: NNSF grant 31500857

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Title: Depression attenuates allodynia and hyperalgesia of neuropathic pain via spinal dorsal horn glucocorticoid receptor.

Authors: *X. WEI[1], F. LUO[2];
[1]Inst. of Psychology,Chinese Acad. of Sci., Beijing City, China; [2]Inst. of Psychology,Chinese Acad. of Sci., Beijing, China

Abstract: Our previous study improved that depression attenuate chronic pain in rats, but the certain neural mechanisms in spinal dorsal horn (SDH) remain to be elucidated. Glucocorticoid receptors (GRs) is a classical nuclear receptor. Recent work demonstrated that GRs' function is disabled in some related brain regions of depressive-like rats. Besides, GRs expression is up-regulates in ipsilateral SDH of neuropathic pain rats. Thus, SDH GRs may play a crucial role in the depressive-like rat with neuropathic pain. This research divided male SD rats into 4 groups: Sham, Olfactory bulbectomy (OB), Spinal nerve ligation (SNL), and OB+SNL, group. First, OB was carried on following the open-field and sucrose consumption baseline tests. 14 days later, SNL or Sham surgery was executed. Mechanical allodynia and thermal hyperalgesia was tested 7 days after SNL. Then, Western Blot was carried out to analyise the expression of GRs in SDH. The locomotive distance and rearing number in the open-field test were significant up-regulated, meanwhile sucrose consumption score was down-regulated after OB. Ipsilateral mechanical paw withdraw threshold (PWT), as well as thermal paw withdraw latency (PWL), of OB+SNL group was up-regulated compared with SNL group. Western blotting results revealed that in OB group

cytoplasm GR level was increased, yet nucleus GR level was decreased (vs. Sham group) ; and in SNL group nucleus GR level was increased (vs. Sham group). What's more, OB+SNL group nucleus GR level was significantly lower than that of SNL group. Pearson correlation analysis revealed that, both mechanical PWT and thermal PWL were negatively correlated with nucleus GR levels. In order to explain the possible neural mechanism, GR selective agonist dexamethasone (Dex) was applied intrathecally on the 15th day following OB operation for 1 week, 4ug daily. Mechanical PWT and thermal PWL of SNL and OB+SNL group were significantly down-regulated by Dex. In SDH, nucleus GR levels were significantly up-regulated by Dex, especially in SNL and OB+SNL group, which were significantly higher than that of Sham and OB group. BDNF plays a crucial role in occurrence and development of neuropathic pain. The effect of OB or/and SNL surgeries on expression of BDNF was similar with that of nuclear GR in SDH. BDNF expression was also up-regulated by Dex, especially in the SNL and OB+SNL group which showed enhancement of mechanical allodynia and thermal hyperalgesia. In conclusion, because of nuclear translocation dysfunction in SDH induced by OB, GRs were not able to regulate their responsive protein such as BDNF, eventually leading to attenuation of neuropathic pain caused by SNL.

Disclosures: X. Wei: None. **F. Luo:** None.

Poster

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Topic: D.01. Sensory Disorders

Support: NIH Grant EY016525

Title: Ngf r100w defines a role of p75ntr in nociception

Authors: *W. YANG[1], K. SUNG[2], C. WU[2], W. MOBLEY[2];
[1]Dept. of Neurology, Ruijin Hosp., Shanghai City, China; [2]Dept. of Neurosciences, Univ. of California at San Diego, SAN DIEGO, CA

Abstract: Nerve growth factor (NGF) exerts multiple functions on target neurons throughout development. The recent discovery of a point mutation in NGF(NGFR100W) in patients with hereditary sensory and autonomic neuropathy, type V (HSAN V) made it possible to distinguish the signaling mechanisms that lead to two functionally different outcomes of NGF: trophic versus nociceptive. We performed extensive biochemical, cellular and live imaging experiments to examine the binding and signaling properties of NGFR100W. Our results show that, similar to

the wildtype NGF (wtNGF), NGFR100W was capable of binding to and activating the TrkA receptor and its downstream signaling pathways to support neuronal survival and differentiation. However, NGFR100W failed to bind and stimulate the 75kD neurotrophic factor receptor (p75NTR)-mediated signaling cascades. Furthermore, NGFR100W no longer had the ability to induce hyper-potentiation of dorsal root ganglion (DRG) sensory neurons as detected by single cell patching clamp assay. Ceramide, a downstream signaling molecule to p75NTR appeared to play a significant role in NGF-induced hyper-sensitization of DRG neurons. Intraplantar injection of NGFR100W into adult rats induced neither thermal nor mechanical hyperalgesia either acutely or chronically. Taken together, our studies provide evidence that NGFR100W retains trophic support capability through TrkA but no longer engages the p75NTR signaling pathways. These results may, at least in part, explain the increase in pain threshold thus a loss of pain perception in HSAN V patients.

Disclosures: W. Yang: None. **K. Sung:** None. **C. wu:** None. **W. Mobley:** None.

Poster

616. Neuropathic Pain II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 616.29/OO1

Topic: D.02. Somatosensation: Pain

Support: Kentucky Spinal Cord and Head Injury Research Trust 09-12A

Kentucky Spinal Cord and Head Injury Research Trust 10-10

Kentucky Spinal Cord Injury Research Center Traineeship

Paralyzed Veterans of America Fellowship #2579

NIH R21 NS080091

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Title: Behavioral, molecular and electrophysiological changes induced by skin incision: implications for the development of chronic pain

Authors: *K. K. RAU[1], C. HILL[3], B. HARRISON[2], G. VENKAT[2], H. KOENIG[1], S. COOK[3], A. RABCHEVSKY[4], B. TAYLOR[4], T. HAI[5], J. PETRUSKA[2];
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KY; [3]Burke Med. Res. Inst., White Plains, NY; [4]Dept. of Physiol., Univ. of Kentucky, Lexington, KY; [5]Dept. of Mol. and Cell. Biochem., Ohio State Univ., Columbus, OH

Abstract: Tissue damage is one of the major etiological factors in the emergence of chronic/persistent pain, although mechanisms remain enigmatic. Using incision of the back skin of adult rats as a model for tissue damage, we observed sensitization in the nociceptive cutaneous trunci muscle reflex enduring to 28 days post-incision (DPI). Previously, we have shown that skin incision induces altered gene regulation in sensory neurons that is maintained long after the skin has healed and is resistant to standard clinical interventions (e.g., nerve block, NSAID). To determine if the enduring behavioral changes we observed with the cutaneous trunci muscle reflex corresponded with a long-term impact of tissue damage on sensory neurons, we examined the temporal expression profile of injury-regulated genes and the electrophysiological properties of traced dorsal root ganglia (DRG) sensory neurons. In this study, the mRNA for the injury/stress-hub gene Activating Transcription Factor 3 (ATF3) was upregulated and peaked within 4 DPI, after which levels declined but remained significantly elevated out to 28 DPI, a time when the initial incision appears healed and tissue-inflammation largely resolved. Accordingly, stereological image analysis indicated that some neurons expressed ATF3 only transiently (mostly medium-large neurons), while in others it was sustained (mostly small neurons), suggesting cell-type-specific responses. In retrogradely-traced ATF3-expressing neurons, Calcium/calmodulin-dependent protein kinase type IV (CAMK4) protein levels and isolectin-B4 (IB4)-binding were suppressed whereas Growth Associated Protein-43 (GAP-43) and Neuropeptide Y (NPY) protein levels were enhanced. Electrophysiological recordings from DiI-traced sensory neurons 28 DPI showed a significant sensitization limited to ATF3-expressing neurons. Thus, ATF3 expression is revealed as a strong predictor of single cells displaying enduring pain-related electrophysiological properties. These findings may represent a new consideration for the impact that tissue damage may have on the health and well-being of individuals and on developing strategies for treatment.

Disclosures: K.K. Rau: None. **C. Hill:** None. **B. Harrison:** None. **G. Venkat:** None. **H. Koenig:** None. **S. Cook:** None. **A. Rabchevsky:** None. **B. Taylor:** None. **T. Hai:** None. **J. Petruska:** None.

Poster

616. Neuropathic Pain II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 616.30/OO2

Topic: D.02. Somatosensation: Pain

Support: Department of Neural and Pain Science, University of Maryland School of Dentistry

Title: Metabolic reprogramming of nociceptors initiated by HIF-1 alpha mediates the transition of pain from acute to chronic

Authors: *O. K. MELEMEDJIAN, Q. J. BANKS;
Dept. of Neural & Pain Sci., Univ. of Maryland Dent. Sch., Baltimore, MD

Abstract: Acute pain is an essential physiological response to injury, allowing for quicker recovery by promoting protection of damaged tissue. In some cases, this acute, protective pain becomes chronic, a debilitating condition which persists after the initial injury has healed. Furthermore, the molecular bases for how chronic pain is initiated and maintained are not well understood. Since metabolism is inextricably linked to every aspect of cellular function and the shift from acute to chronic pain would require metabolic changes that can maintain the chronic pain state, we hypothesized that metabolic reprogramming leads to the transition of acute pain to chronic. Utilizing nerve growth factor (NGF)-induced hyperalgesic priming, we tested this hypothesis. Intraplantar injection of NGF evokes tactile hypersensitivity that resolves within 72 hours. However, the animals that received NGF become primed for developing prolonged hypersensitivity following the intraplantar administration of prostaglandin E2 (PGE_2). We determined that NGF triggers local translation of the transcription factor hypoxia-inducible factor 1-alpha (HIF-1 alpha) which is retrogradely transported to the soma of sensory neurons where it initiates the reprogramming of cellular metabolism. Using Extracellular flux analysis, we determined that pain is associated with a distinct metabolic phenotype where sensory neurons display increased glycolysis and reduced oxidative phosphorylation. Moreover, during the primed phase the animals do not display tactile hypersensitivity and the sensory neurons exhibit normal oxidative phosphorylation. Crucially, reversing these metabolic changes alleviates pain. Hence, these findings provide novel insights into the role of metabolic reprogramming of nociceptors in the development and maintenance of chronic pain.

Disclosures: O.K. Melemedjian: None. **Q.J. Banks:** None.

Poster

617. Pain: Non-Opioid Analgesics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 617.01/OO3

Topic: D.02. Somatosensation: Pain

Support: KAKENHI(15K14328)

Title: Small-animal neuroimaging analysis of placebo analgesia

Authors: ***Y. L. CUI**[1], Y. ZENG[1,2], Q. ZENG[2], D. HU[1], W. YANG[3], E. HAYASHINAKA[1], Y. WADA[1], Y. WATANABE[1];
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[2]Bioelectromagnetics Laboratory, Sch. of Medicine,, [3]Dept. of Neurobiology, Sch. of Medicine,, Zhejiang Univ., Hangzhou, China

Abstract: Placebo analgesia is a kind of pain relief that follows the administration of an inert treatment and is thought to result from expectation and implication. Placebo analgesia is a high-order neuropsychological process that activate intrinsic descending pain control system. Neuroimaging studies in human implicate a hierarchical recurrent system is involved in placebo analgesia, including cortical (prefrontal cortex), subcortical (amygdala), midbrain (periaqueductal gray), medulla (rostral ventromedial medulla), and spinal cord. However, the underlying neural and molecular mechanisms in detail are still poorly understood. To address this issue, we tried to establish an animal model of placebo analgesia in neuropathic pain rats using Pavlovian conditioning, in which neuropathic pain was caused by tight ligation of the lumber 5/6 spinal nerves (SNL) and Pavlovian conditioning was established with a painkiller (gabapentin) as the unconditioned stimulus. We show here that after a few trials of paired stimulation of gabapentin with conditioning stimulus, the inert treatment (saline, i.p.) alone also induced analgesia in some animals. Such placebo analgesia was completely blocked by naloxone (i.p., 5mg/kg), a mu-opioid receptor antagonist. In order to identify the brain regions involved in placebo analgesia, we also assessed the regional brain activity in these animal using small-animal neuroimaging method combing 2-deoxy-2-[^{18}F]fluoro-D-glucose positron emission tomography (PET) imaging with statistical parametric mapping analysis. Small-animal neuroimaging results showed that regional brain activity was increased in the medial prefrontal cortex and anterior insular cortex, and suppressed in the primary somatosensory cortex and primary motor cortex in the placebo analgesia group as compared with naloxone group. These results suggest that placebo analgesia could be established in animals using Pavlovian conditioning and that the brain regions identified in the present study could be involved in placebo analgesia.

Disclosures: **Y.L. Cui:** None. **Y. Zeng:** None. **Q. Zeng:** None. **D. Hu:** None. **W. Yang:** None. **E. Hayashinaka:** None. **Y. Wada:** None. **Y. Watanabe:** None.

Poster

617. Pain: Non-Opioid Analgesics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 617.02/OO4

Topic: D.02. Somatosensation: Pain

Title: *In vivo* characterization of a selective monoacylglycerol lipase inhibitor ABD-1970 in rodents and activity in models of pain

Authors: *J. R. CLAPPER, J. L. BLANKMAN, A. R. COPPOLA, A. KNIZE, G. SIMON, J. CISAR, O. WEBER, M. NIPHAKIS, C. HENRY, I. FRASER, C. GRICE, A. EZEKOWITZ, G. O'NEILL;
Abide Therapeut., San Diego, CA

Abstract: Monoacylglycerol lipase (MGLL) is the primary regulator of 2-arachidonoylglycerol (2-AG) an endogenous ligand of the cannabinoid (CB) receptors CB1 and CB2. Selective blockade of MGLL inhibits the hydrolytic degradation of 2-AG resulting in elevated 2-AG levels in the CNS and periphery, and CB1/2-dependent anti-nociceptive and anti-inflammatory effects. In the rodent brain and select peripheral tissues, MGLL inactivation also reduces levels of the catabolic product of 2-AG hydrolysis, arachidonic acid, a metabolic precursor to pro-inflammatory prostanoids. Here, we present a comprehensive in vivo characterization of a selective covalent inhibitor of MGLL, ABD-1970. ABD-1970 is orally active in rodents producing a rapid and potent inhibition of MGLL and elevation of 2-AG in the brain. In vivo blockade of 2-AG hydrolysis was also associated with reduced levels of arachidonic acid and prostanoids PGE_2, PGD_2 and $PGF_2\alpha$ in the brain. Using activity-based protein profiling to comprehensively profile the in vivo selectivity of ABD-1970 against other serine hydrolases, α/β-hydrolase domain-containing 6 (ABHD6) and rodent-specific carboxylesterase 1c (CES1c) were the only off-targets identified, albeit at doses above those required for in vivo pharmacological activity at MGLL. In time course experiments, MGLL activity was inversely correlated with ABD-1970 concentrations in the blood and brain with recovery of MGLL activity observed as the compound was eliminated from the body. Furthermore, ABD-1970 was efficacious in the rat incisional model of acute post-operative pain, the rat formalin and complete Freund's adjuvant models of inflammatory pain and the rat chronic constriction injury model of neuropathic pain. In the formalin model, ABD-1970 produced enhanced activity in combination with standard of care agents, pregabalin and morphine. Integrated analysis of compound exposure, brain target engagement, and 2-AG levels reveal a strong relationship between PK, central biomarkers and efficacy in models of pain. Importantly, acute administration of ABD-1970 lacked effects on total exploratory activity in an open field test. These results identify ABD-1970 as a highly potent and selective in vivo active inhibitor of MGLL and underscore the therapeutic potential of selective MGLL inhibitors for the treatment of acute and chronic pain.

Disclosures: **J.R. Clapper:** A. Employment/Salary (full or part-time): Abide Therapeutics. **J.L. Blankman:** A. Employment/Salary (full or part-time): Abide Therapeutics. **A.R. Coppola:** A. Employment/Salary (full or part-time): Abide Therapeutics. **A. Knize:** A. Employment/Salary (full or part-time): Abide Therapeutics. **G. Simon:** A. Employment/Salary (full or part-time): Abide Therapeutics. **J. Cisar:** A. Employment/Salary (full or part-time): Abide Therapeutics. **O. Weber:** A. Employment/Salary (full or part-time): Abide Therapeutics. **M. Niphakis:** A. Employment/Salary (full or part-time): Abide Therapeutics. **C. Henry:** A. Employment/Salary (full or part-time): Abide Therapeutics. **I. Fraser:** A. Employment/Salary (full or part-time):

Abide Therapeutics. **C. Grice:** A. Employment/Salary (full or part-time): Abide Therapeutics. **A. Ezekowitz:** A. Employment/Salary (full or part-time): Abide Therapeutics. **G. O'Neill:** A. Employment/Salary (full or part-time): Abide Therapeutics.

Poster

617. Pain: Non-Opioid Analgesics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 617.03/OO5

Topic: D.02. Somatosensation: Pain

Title: *In vitro* characterization of a selective monoacylglycerol lipase inhibitor ABD-1970 in human systems

Authors: *J. L. BLANKMAN, C. HENRY, A. KNIZE, G. SIMON, J. CISAR, O. WEBER, M. NIPHAKIS, J. R. CLAPPER, A. COPPOLA, I. FRASER, C. GRICE, A. EZEKOWITZ, G. O'NEILL;
Abide Theraputics, San Diego, CA

Abstract: Monoacylglycerol lipase (MGLL) is a serine hydrolase enzyme that converts monoacylglycerols into fatty acids and glycerol. The MGLL substrate 2-arachidonoylglycerol (2-AG) is an endogenous ligand of the cannabinoid receptors CB1 and CB2, which are the molecular targets of the psychoactive component of *Cannabis sativa,* Δ^9tetrahydrocannabinol (THC). Inhibition or genetic inactivation of MGLL results in accumulation of 2-AG in the brain and periphery and CB1/2-dependent anti-nociceptive and anti-inflammatory effects. Additionally, MGLL inactivation in mice reduces levels of arachidonic acid (AA) and pro-inflammatory prostanoid metabolites in a subset of tissues including the brain, liver and lung. MGLL inhibition may therefore provide therapeutic benefit via enhancing cannabinoid receptor signaling and reducing prostanoid signaling.
To further evaluate the therapeutic potential of MGLL inhibition, we have developed ABD-1970, a potent and selective MGLL inhibitor. ABD-1970 is a hexafluoroisopropyl carbamate that covalently inhibits MGLL in vitro with IC_{50} values of <50 nM across species. The selectivity of ABD-1970 for MGLL amongst other serine hydrolases has been extensively profiled using gel- and mass spectrometry-based activity-based protein profiling (ABPP) technologies.
ABD-1970 has been used to explore the effects of MGLL inhibition in human systems in vitro. We determined that MGLL is expressed and active in many regions of the post-mortem human brain. Homogenates prepared from human brain cortex readily hydrolyze 2-AG and we confirmed that this activity is largely mediated by MGLL by its sensitivity to ABD-1970 treatment.

Though MGLL inhibition has been shown to reduce prostanoid production in mice, the effects of MGLL inhibition on prostanoid production and signaling in human systems is not well understood. Cyclooxygenase (COX) inhibitors, such as non-steroidal anti-inflammatory drugs (NSAIDs), dramatically reduce prostanoid production in humans and carry both therapeutically beneficial effects as well as gastro-intestinal and cardiovascular side-effects. To compare the effects of MGLL and COX inhibition in human cells, ABD-1970 and the NSAID indomethacin were tested in three paradigms: 1) prostanoid production in stimulated human whole blood, 2) prostanoid production in stimulated human endothelial cells and 3) collagen-induced platelet aggregation. The results of these assays clearly differentiated MGLL inhibition from COX inhibition as ABD-1970 was without effect in all three assays whereas indomethacin produced robust suppression of prostanoid production and platelet aggregation.

Disclosures: **J.L. Blankman:** A. Employment/Salary (full or part-time): Abide Therapeutics. **C. Henry:** A. Employment/Salary (full or part-time): Abide Therapeutics. **A. Knize:** A. Employment/Salary (full or part-time): Abide Therapeutics. **G. Simon:** A. Employment/Salary (full or part-time): Abide Therapeutics. **J. Cisar:** A. Employment/Salary (full or part-time): Abide Therapeutics. **O. Weber:** A. Employment/Salary (full or part-time): Abide Therapeutics. **M. Niphakis:** A. Employment/Salary (full or part-time): Abide Therapeutics. **J.R. Clapper:** A. Employment/Salary (full or part-time): Abide Therapeutics. **A. Coppola:** A. Employment/Salary (full or part-time): Abide Therapeutics. **I. Fraser:** A. Employment/Salary (full or part-time): Abide Therapeutics. **C. Grice:** A. Employment/Salary (full or part-time): Abide Therapeutics. **A. Ezekowitz:** A. Employment/Salary (full or part-time): Abide Therapeutics. **G. O'Neill:** A. Employment/Salary (full or part-time): Abide Therapeutics.

Poster

617. Pain: Non-Opioid Analgesics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 617.04/OO6

Topic: D.02. Somatosensation: Pain

Support: NIDA Grant DA016644

Title: A sex comparison of cannabidiol-tetrahydrocannabinol interactions on antinociception

Authors: *S. C. BRITCH, R. M. CRAFT;
Psychology, Washington State Univ., Pullman, WA

Abstract: The current study investigated sex differences in cannabidiol (CBD)-tetrahydrocannabinol (THC) interactions on antinociception and locomotor activity in rats.

Experiment 1 tested the hypothesis that CBD enhances THC-induced antinociception on tests of acute pain. A single dose of CBD (0, 10 or 30 mg/kg i.p.) was administered 15 min before a single dose of THC (0, 1.8, 3.2, 5.6 or 10 mg/kg i.p.). Rats were tested for antinociception on warm water tail withdrawal and paw pressure tests at 15, 30, 60, 120, 240 and 360 min post-THC injection. A 10-min locomotor activity test was also completed after nociceptive testing at each time point. Experiment 2 tested the hypothesis that a long CBD pretreatment time would enhance THC's effects more than a short pretreatment time. CBD 0 or 30 mg/kg was injected 13 hr or 15 min before THC 0 or 1.8 mg/kg, and rats were tested at 30, 60, 120, 240, 360 and 480 min post-THC injection, using the same behavioral tests as in Experiment 1. In both Experiment 1 & 2, THC caused dose- and time- dependent antinociception that was significantly greater in females than males. THC also caused dose- and time-dependent hypolocomotion, with no consistent sex differences. CBD enhanced THC-induced antinociception at later time points (240 & 360 min) on both nociceptive tests. In addition, CBD enhanced THC-induced hypolocomotion at 240 & 360 min, although when given alone, CBD increased locomotion at these later time points. CBD modulation of THC's effects was not sex-dependent on any measure. In Experiment 2, CBD-THC interactions did not consistently differ between rats tested with the long vs. short CBD pretreatment times. The present results suggest that CBD can slightly enhance THC-induced antinociception and hypolocomotion long after administration in both sexes, but the drug interaction was more robust on locomotion than on antinociception.

Disclosures: S.C. Britch: None. **R.M. Craft:** None.

Poster

617. Pain: Non-Opioid Analgesics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 617.05/DP07 (Dynamic Poster)

Topic: D.02. Somatosensation: Pain

Support: U.S. Army Institute of Surgical Research

US Army Medical Research and Material Command (MRMC) Combat Casualty Care Research Program

MRMC Clinical and Rehabilitative Medicine Research Program

Title: Curcumin is an effective analgesic for burn pain: evidence from animal and human tissue based experiments

Authors: *J. L. CLIFFORD[1], B. P. CHEPPUDIRA[2], A. TREVINO[2], A. GREER[2], M. M. SALAS[2];
[1]US Army Ctr. For Envrn. Hlth. Res., Frederick, MD; [2]US Army Inst. for Surgical Res., San Antonio, TX

Abstract: Recovery and healing from severe burn injuries involves intensely painful procedures including wound debridement, dressing changes, and strenuous physical and occupational therapy. Although opioids are available to treat severe pain, a multitude of side effects including development of tolerance and dependence, accompany routine use of opioids for severe pain. In order to reduce the amount of opioids necessary for burn patients, alternative analgesics with reduced side effect profiles deserve exploration. Curcumin, a major constituent of turmeric (*Curcuma longa*), which has been utilized medicinally for centuries, has potential as an analgesic. Although curcumin is well tolerated for human consumption, its use as an analgesic in burn wound care has not yet been studied. We have assessed the effect of curcumin treatment (100uM, 24 hr) on the activity of several inflammatory signaling mediators in heat shocked (20 min, 45C or 55C) cultured HaCaT, human keratinocyte-derived cells. Curcumin treatment suppressed baseline phosphorylation levels of NF-kB and the heat shock induced phosphorylation of p38 MAPK. We also determined the effect of locally administered curcumin (SC injection, 100ug in 100ul vehicle, daily for 5 days) on two different types of pain behavior, mechanical allodynia and thermal hyperalgesia, in a full thickness thermal injury (FTTI) rat hindpaw pain model. Curcumin attenuated thermal hyperalgesia after the 2nd day of injections and attenuated mechanical allodynia after the 4th day of injections. Curcumin did not elicit any analgesic effects on the contralateral (non-injured) hindpaw; indicating it does not have CNS mediated effects on nociception. Finally, we have determined the effect of curcumin on pain signaling in human peripheral nerve tissue using a unique dental pulp in-vitro assay system. Curcumin pre-treatment (20 min., 100ug/100ul vehicle) suppressed the release of the pain neurotransmitter calcitonin gene-related peptide (CGRP) which is caused by treatment with capsaicin. These results indicate that curcumin has potential as an analgesic for burn pain management, and could reduce the amount of opioids administered to burn patients.

Disclosures: J.L. Clifford: None. **B.P. Cheppudira:** None. **A. Trevino:** None. **A. Greer:** None. **M.M. Salas:** None.

Poster

617. Pain: Non-Opioid Analgesics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 617.06/OO7

Topic: D.02. Somatosensation: Pain

Title: Impact anesthesics consumpsion of pacients in protocol the kidney transplant with m-entropy monitoring backgroud

Authors: *C. SORIA-FREGOZO[1], A. CASTELLANOS ALVARADO[2], M. MIRANDA BELTRAN[2], A. ZEPEDA GONZALEZ[3], I. GONZALEZ HERNADEZ[3], M. PEREZ VEGA[2]; [1]Univ. de Guadalajara, Lagos de Moreno Jalisco, Mexico; [2]Univ. de Guadalajara, Guadalajara, Mexico; [3]Inst. Mexica del Seguro Social, Guadalajara, Mexico

Abstract: More than 500 million of persons in the word present kidney failure. In Mexico the kidney injury is of the principal cause of hospital attention. In Hospital of Specialty of CMO, IMSS is performer around of 13,000 general anesthesia in patients for year and is performer a proximity 160 kidney transplants of towards living donor kidney. The patients with injury kidney need surgery under general anesthesia frequently, and not exist a protocol that permit optimize the anesthesic consumption and see the impact with the M-entropy monitoring in the patients with kidney transplant. Impact in the anesthesics consumption of patients in protocol of kidney transplant with M-Entropy monitoring *vs* clinical variables simple monitoring. One hundred four patients were enrolled in this study, of protocol of patients reaccept of kidney transplant and towards living donor kidney with M-entropy monitoring and clinical variables simple monitoring; 22 patients donors with entropy monitoring (DE) group, 27 patients donors with clinical variables simple monitoring (DH) group, 23 patients reaccepts of kidney transplant with entropy monitoring (RE) group and 31 patients reaccept of kidney transplant with clinical variables simple monitoring (RH) group. In the premedication of the anesthesia, the fentanil consumption was significantly less in the DE group *vs* with the DH group and this was less in the group RE *vs* with RH group. Midazolam consumption was similar in allgroups. During the induction of the anesthesia the fentanil consumption was significantly less in the RE group *vs* RH group and in the maintenance of the anesthesia the fentanil consumption was significantly less in the RE group *vs* RH group and the fentanil consumption was less DE group *vs* DH group . In the induction of the anesthesia the propofol consumption was less in the DE group *vs* DH group. In the maintenance of the anesthesia, the desfluarano consumption was less in the DE and RE groups. The use with M-Entropy monitoring impact in anesthesics consumption in the groups of patients with kidney injury with entropy monitoring *vs* with clinical variables simple monitoring, decreased thecost, anesthesia awareness and in the quality of the attention of patients in protocol of kidney transplant.

Disclosures: C. Soria-Fregozo: None. **A. Castellanos Alvarado:** None. **M. Miranda Beltran:** None. **A. Zepeda Gonzalez:** None. **I. Gonzalez Hernadez:** None. **M. Perez Vega:** None.

Poster

617. Pain: Non-Opioid Analgesics

Location: Halls B-H

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Topic: D.02. Somatosensation: Pain

Support: DA037673 (to AGH)

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EY024717 (to GAT)

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Title: Positive allosteric modulation of cannabinoid CB1 receptor signaling suppresses chemotherapy-induced peripheral neuropathy

Authors: *R. SLIVICKI[1], P. KULKARNI[2], K. MACKIE[1], G. THAKUR[2], A. HOHMANN[1]; [1]Indiana Univ., Bloomington, IN; [2]Bouvé Col. of Hlth. Sci., Northeastern Univ., Boston, MA

Abstract: Activation of cannabinoid CB1 receptors either directly (via agonistic action) or indirectly (elevation of endocannabinoid tone through inhibitors of endocannabinoid deactivation) suppresses pathological pain. However, unwanted central 'side' effects (e.g. psychoactivity, tolerance) constrain therapeutic dosing. We hypothesized that positive allosteric modulation of CB1 receptor signaling would suppress neuropathic pain produced by chemotherapy treatment while bypassing cardinal signs associated with direct CB1 receptor activation. In addition, we sought to examine the potential for synergistic interactions between positive allosteric modulation of CB1 and inhibitors of endocannabinoid deactivation. We, therefore, compared the therapeutic efficacy of GAT211, a positive allosteric modulator of CB1 receptor signaling, with URB597, an inhibitor of fatty-acid amide hydrolase (FAAH), and JZL184, an inhibitor of monoacylglycerol lipase (MGL) using a mouse model of chemotherapy-induced peripheral neuropathy. Within-subjects dose-response curves were constructed for GAT211, URB597, and JZL184; ED_{50} values were generated and used in fixed combinations to determine if the combination treatments produced antinociceptive effects that were additive or synergistic. GAT211 produced a CB1-dependent suppression of mechanical and cold allodynia induced by the taxane chemotherapeutic agent paclitaxel. Isobolographic analysis revealed synergistic interaction of GAT211 with inhibitors of either FAAH or MGL. Moreover, GAT211 did not produce cardinal signs of cannabinoid receptor activation (hypothermia, motor ataxia, catalepsy, tail flick antinociception) in wildtype mice but produced signs of catalepsy in transgenic mice lacking either FAAH or MGL. Therapeutic efficacy of GAT211 was preserved

over a dosing period of 19 days with no appreciable signs of tolerance developing to its antinociceptive efficacy. By contrast, JZL184 initially displayed therapeutic efficacy but tolerance developed with repeated dosing. Thus, positive allosteric modulation of CB1 represents a promising strategy for harnessing the therapeutic potential of the endocannabinoid signaling system to suppress neuropathic pain without producing tolerance to therapeutic efficacy or detrimental CNS side-effects observed with orthosteric CB1 agonists. Moreover, our studies also suggest that CB1 positive allosteric modulators may show greater therapeutic potential compared to sustained inhibition of MGL, as manifested by absence of both tolerance and cardinal signs of CB1 intoxication.

Disclosures: R. Slivicki: None. **P. Kulkarni:** None. **K. Mackie:** None. **G. Thakur:** None. **A. Hohmann:** None.

Poster

617. Pain: Non-Opioid Analgesics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 617.08/OO9

Topic: D.02. Somatosensation: Pain

Title: Bisphenol A inhibits nerve conduction in the frog sciatic nerve with an efficacy comparable to those of local anesthetics

Authors: N. MAGORI[1], *K. MIZUTA[2,1], T. FUJITA[1], H. YAMAGATA[1], C. WANG[1], R. HIRAO[1], R. SUZUKI[1], E. KUMAMOTO[1];
[1]Saga Med. Sch., Saga, Japan; [2]RIKEN Brain Sci. Inst. - Wako, Saitama, Japan

Abstract: Bisphenol A (BPA) is a monomer component in chemical products such as polycarbonate plastics and thus the products including BPA are widely used in various materials including food packaging. Owing to a lipophilic property of BPA, BPA is easily absorbed into the human body through food and drink in contact with materials containing BPA. Since BPA has an estrogenic action albeit this activity is comparatively weak, BPA interferes with normal endocrine function as an endocrine-disrupting chemical and therefore exerts a toxicological action on animals. BPA affects not only the endocrine system but also various physiological functions including nerve activity. Although BPA is reported to inhibit nerve conduction, this action has not yet been examined fully. In order to know a detail of the inhibition, we examined the effect of BPA on compound action potentials (CAPs) recorded from the frog sciatic nerve by using the air-gap method. Since BPA has been reported to bind to a local anesthetic (LA) receptor site on human cardiac Na^+ channels, the BPA result was compared with those of LAs. Treatment of the sciatic nerve with BPA (0.5 mM) for 20 min resulted in a decrease in CAP peak

amplitude to about 40 % of control. The CAP amplitude did not recover to control for up to 1 hour, when this amplitude was about 65 % of control, in nerves returned to drug-free solution. The CAP peak amplitude reduction produced by BPA was concentration-dependent with a half-maximal inhibitory concentration (IC_{50}) value of 0.31 mM. The BPA activity was resistant to an estrogen-receptor antagonist 4-hydroxytamoxifen. 4-Hydroxytamoxifen itself reduced CAP peak amplitude with the IC_{50} value of 0.26 mM, a value comparable to that of BPA. A natural estrogen 17β-estradiol at a maximally dissolvable concentration (0.05 mM) had an action similar to that of BPA. LAs (prilocaine and pramoxine) inhibited CAPs in a concentration-dependent manner. When compared with these and available LA data in the frog sciatic nerve, the efficacy of BPA in inhibiting CAPs was similar to those of pramoxine, ropivacaine and levobupivacaine (0.21, 0.34 and 0.23 mM, respectively), while being larger than those of prilocaine, procaine, lidocaine and cocaine (IC_{50} = 1.8, 2.3, 0.74 and 0.80 mM, respectively) and smaller than that of tetracaine (0.014 mM). In conclusion, BPA has an ability to inhibit nerve conduction in a manner independent of estrogen receptors with an efficacy comparable to those of some LAs. Such a conduction inhibition could contribute to at least a part of the effect of BPA on the nervous system.

Disclosures: **N. Magori:** None. **K. Mizuta:** None. **T. Fujita:** None. **H. Yamagata:** None. **C. Wang:** None. **R. Hirao:** None. **R. Suzuki:** None. **E. Kumamoto:** None.

Poster

617. Pain: Non-Opioid Analgesics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 617.09/OO10

Topic: D.02. Somatosensation: Pain

Support: Career Development Award-University of Arizona

Startup Funds. University of Arizona

Title: Antinociceptive effects of light therapy on acute and chronic pain models

Authors: ***M. M. IBRAHIM**[1,2], K. B. GILBRAITH[3], A. MOUTAL[2], X. YANG[2], L. CHEW[2], T. MILNES-LARGENT[2], F. PORRECA[2,3], T. P. MALAN[3,4], T. W. VANDERAH[2,3], A. PATWARDHAN[3,2], R. KHANNA[2];
[1]Univ. of Arizona Dept. of Anesthesiol., Tucson, AZ; [2]Pharmacol., [3]Anesthesiol., Univ. of Arizona, Tucson, AZ; [4]Pharmacol., university of Arizona, Tucson, AZ

Abstract: Managing chronic pain is challenging. Opioids are commonly prescribed for chronic pain despite weak evidence for long-term efficacy. The Centers for Disease Control and Prevention recommend non-opioid therapy for chronic pain. While some evidence points to light therapy being beneficial in certain medical conditions, this approach remains to be explored for acute and chronic pain. Here, we investigated the possible antinociceptive effects of several light emitting diodes (LED), in the visible spectrum, on naïve and neuropathic pain rats. Green LED (wavelength 525 nanometers) exposure for eight hours for five days increased withdrawal latency to a noxious thermal stimulus, which persisted for four days following termination of last exposure. The antinociception was mediated via actions on central mu-opioid receptor pathways unrelated to stress. No apparent side-effects were noted and motor performance was not impaired. Blocking pain-modulatory pathways by inactivation of the rostral ventromedial medulla prevented expression of light-induced antinociception. Antinociception was prevented by opaque contacts despite LED exposure or by green contacts exposed to room light, but not in rats with pigmentation arguing for a role of the visual system. Pharmacological and proteomic profiling of dorsal root ganglion (DRG) neurons from green-LED exposed rats identified changes in calcium channel activity, including a decrease in the N-type (CaV2.2) channel, a primary analgesic target. Tetrodotoxin-sensitive and -insensitive sodium currents in DRGs were unchanged by green-light exposure. Finally, green-LED exposure reversed thermal and mechanical hyperalgesia in rats with spinal nerve ligation or injection of envelope glycoprotein 120 of HIV-1. Thus, green-LED therapy represents a novel, non-pharmacological approach for managing chronic pain.

Disclosures: M.M. Ibrahim: None. **K.B. Gilbraith:** None. **A. Moutal:** None. **X. Yang:** None. **L. Chew:** None. **T. Milnes-Largent:** None. **F. Porreca:** None. **T.P. Malan:** None. **T.W. Vanderah:** None. **A. Patwardhan:** None. **R. Khanna:** None.

Poster

617. Pain: Non-Opioid Analgesics

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Program#/Poster#: 617.10/OO11

Topic: D.02. Somatosensation: Pain

Support: SIP20161162

Title: Pharmacological profile of LIA a novel analogue of lidocaine

Authors: *M. DECIGA-CAMPOS[1], F. J. LÓPEZ-MUÑOZ[2];
[1]Escuela Superior De Medicina IPN, D.F. Mexico, Mexico; [2]Farmacobiología, Inst. Politécnico Nacional, CINVESTAV-Sur, Mexico

Abstract: N-(2,6-dichlorophenyl)-2-(4-methyl-1-piperidinyl)acetamide (LIA), a lidocaine analogue, has potential applications in treating neuropathic pain. The aim of this work was to characterize the pharmacological activity of LIA related with central nervous system activity. Anesthetic effect was tested in guinea pigs and mice. Ambulatory activity, anti-anxiety effect, sodium pentobarbital (PB)-induced hypnosis and pentylenetetrazol (PTZ)-induced seizures test were evaluated in mice to determine the possible central nervous system activity. LIA (2%) presents, similar to lidocaine (2%), anesthetic activity on the corneal reflex, infiltration anesthesia and tail immersion test. LIA (1-100 mg/Kg, i.p.), similar to lidocaine (1-100 mg/Kg, i.p.), presents a dose-dependent sedative-hypnotic effect in mice. Both compounds did not produce anti-anxiety activity in mice. LIA did not prevent PTZ-induced seizures. However, LIA itself did not produce seizures at high doses in mice, as lidocaine does. High doses of lidocaine produce seizures and vasoconstriction. In this study, we found that LIA shares a similar pharmacological profile as lidocaine´s but without the primary adverse effects of seizures.

Disclosures: M. Deciga-Campos: None. **F.J. López-Muñoz:** None.

Poster

617. Pain: Non-Opioid Analgesics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 617.11/OO12

Topic: D.02. Somatosensation: Pain

Title: Compound action potential inhibition by various antidepressants in the frog sciatic nerve

Authors: R. HIRAO[1], T. FUJITA[1], A. SAKAI[1], C. WANG[1], R. SUZUKI[1], N. MAGORI[1], *M. ISHIMATSU[2], E. KUMAMOTO[1];
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Abstract: An inhibition of action potentials conducting on nerve fibers possibly contributes to at least a part of antinociception produced by analgesics and their adjuvants. We have previously reported the inhibitory effects of a variety of drugs involved in antinociception on fast-conducting and voltage-gated Na^+-channel blocker tetrodotoxin-sensitive compound action potential (CAP) recorded from the frog sciatic nerve. Among the drugs, there are opioids, local anesthetics, α_2-adrenoceptor agonists and anticonvulsants. The aim of the present study was to examine the effects of various antidepressants on frog CAP and then to compare their results obtained with those of analgesics and their adjuvants. The experiments were performed by applying the air-gap method to the sciatic nerve isolated from frogs. Duloxetine, fluoxetine [serotonin and norepinephrine reuptake inhibitor (SNRI) and selective serotonin reuptake inhibitor (SSRI), respectively], amitriptyline and desipramine (tricyclic antidepressants; tertiary

and secondary amines, respectively) reduced the peak amplitude of the CAP with the half-maximal inhibitory concentration (IC_{50}) values of 0.39, 1.5, 0.16 and 1.4 mM, respectively. The duloxetine value was similar to those of anticonvulsants (lamotrigine and carbamazepine), a local anesthetic ropivacaine and an α_2-adrenoceptor agonist dexmedetomidine (0.44, 0.50, 0.34 and 0.40 mM, respectively) while the amitriptyline value was close to those of local anesthetics levobupivacaine and pramoxine (0.23 and 0.21 mM, respectively). The fluoxetine and desipramine values were similar to those of local anesthetics (lidocaine, cocaine and prilocaine: 0.74, 0.80 and 1.8 mM, respectively). They were larger than that of a local anesthetic tetracaine (0.013 mM) while being smaller than those of opioids. We have previously reported that a μ-opioid receptor agonist tramadol and ethylmorphine have the IC_{50} values of 2.3 and 4.6 mM, respectively; morphine and codeine (5 mM each) reduce CAP peak amplitude by only 15 and 30 %, respectively. In conclusion, the four antidepressants inhibited CAPs with efficacies comparable to those of some anticonvulsants and local anesthetics and also of α_2-adrenoceptor agonist, and with more effectiveness than those of opioids. It is suggested that these antidepressants have an ability comparable to those of some anticonvulsants, local anesthetics and α_2-adrenoceptor agonist in inhibiting nerve conduction.

Disclosures: **R. Hirao:** None. **T. Fujita:** None. **A. Sakai:** None. **C. Wang:** None. **R. Suzuki:** None. **N. Magori:** None. **M. Ishimatsu:** None. **E. Kumamoto:** None.

Poster

617. Pain: Non-Opioid Analgesics

Location: Halls B-H

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Program#/Poster#: 617.12/OO13

Topic: D.02. Somatosensation: Pain

Support: TWU Research Enhancement Grant

TWU Chancellor's Research Fellowship

Title: Latex extract of Euphorbia bicolor displays nociceptive and antinociceptive properties in cultured sensory neurons and evokes pain behaviors in rats

Authors: *P. BASU, C. MAIER, D. L. AVERITT;
Biol., Texas Woman's Univ., Denton, TX

Abstract: Opioid-based narcotics are a major component of pain management, but are problematic due to negative side effects mediated by the central nervous system. Pain management can be optimized through discovery of potent non-opioid therapeutics, as well as

targeting the peripheral nervous system. A subpopulation of sensory neurons expresses the transient receptor potential V1 ion channel (TRPV1), gated by capsaicin and noxious heat. TRPV1 activation induces release of proinflammatory peptides, including calcitonin gene-related peptide (CGRP), contributing to peripheral sensitization and hyperalgesia. Potent agonists targeting TRPV1, such as capsaicin and resiniferatoxin, produce peripheral analgesia via TRPV1 desensitization and temporary ablation of nociceptors. Resiniferatoxin is extracted from the latex of *Euphorbia resinifera.* In the same family but native to the Southern United States, *Euphorbia bicolor* shares similar phytochemicals including the irritant *Euphorbium* and thus may share nociceptive and analgesic properties. We hypothesized that *E. bicolor* latex would induce a transient increase in CGRP release from sensory neurons, attenuate capsaicin-evoked CGRP release, and evoke nociception. Rats were decapitated and trigeminal ganglia removed, dissociated, and cultured for 5 days. Cells were washed and treated with buffer for 15 minutes. Fractions were collected as basal release and the cells were then treated with *E. bicolor* latex (0, 12.5, 25, 50, 100, 300 μg/mL) for 15 minutes followed by stimulation with capsaicin (50 nM). CGRP was quantified by ELISA. Another set of rats received an intraplantar injection of *E. bicolor* latex (0, 25, 50, 100, 300, 500 μg/ml) and thermal hyperalgesia and mechanical allodynia were examined at 20, 40, 60 min, 2, and 4 hr post-injection. Here we report that *in vitro E. bicolor* latex induced a twofold increase in CGRP release from sensory neurons similar to capsaicin stimulation. Furthermore, capsaicin-stimulated release was significantly reduced following 50 and 100 μg/mL of latex treatment. *In vivo, E. bicolor* latex evoked significant thermal hyperalgesia and/or mechanical allodynia within 20 min at the 50-500 μg/ml concentrations. There was no significant difference in thermal or mechanical sensitivity in males compared to females. Our data indicate that *E. bicolor* latex is an irritant that displays nociceptive properties *in vivo*, and displays nociceptive and antinociceptive properties *in vitro*. Current studies are ongoing to analyze latex content by HPLC and to determine whether *E. bicolor* displays analgesic properties potentially via the TRPV1 channel, similar to its relative *E. resinifera*.

Disclosures: P. Basu: None. **C. Maier:** None. **D.L. Averitt:** None.

Poster

618. Receptive Field and Response Properties of Tactile Sensation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 618.01/OO14

Topic: D.03. Somatosensation: Touch

Support: DARPA HAPTIX

NSF IGERT

Title: Creating a localized and dynamic somatotopic map of area 3b using cutaneous vibratory stimulation.

Authors: *J. C. TANNER, T. HEARN, S. HELMS TILLERY;
SBHSE, Arizona State Univ., Tempe, AZ

Abstract: Somatosensory area 3b receives input encoding tactile sensations possessing small receptive fields. Area 3b is vital in fine motor feedback tasks and localizing discrete sensation. However, a majority of area 3b exists in the postcentral gyrus' anterior bank. Intrasulcal implantation poses hazards to vasculature and cortical health. To avoid damage, we used penetrating arrays with electrodes on multiple shanks, staggered between shanks, to record parallel and orthogonal to the bank's surface. Using such arrays, we aim to create a somatotopic face map, assess cutaneous response to frequency modulated stimulation, and gauge electrode viability. Analysis and results should translate to other cutaneous maps and activities, such as the grasping or digital manipulation.

Two 16-channel Modular Bionics N-Form electrode arrays (2x2 shank structure with four electrodes per shank at custom depths) were implanted in the facial representation of somatosensory cortex in two Maccaca mullata. Electrode connections were housed in a laser sintered chamber designed to lie flush with the non-human primates' (NHPs') cranium. Frequency modulated vibratory stimulation was delivered to eight locations on the NHPs' face contralateral to the implant. Neural recordings were performed using a Ripple Grapevine Neural Interface System and custom MATLAB software. Vibratory stimulation was achieved using a custom device controlled by MATLAB software. Stimulation force was consistently between 2 and 10 mN, delivered at 10 Hz to 130 Hz on 20 Hz intervals.

Electrodes were implanted without difficulty into somatosensory cortex, providing stable signals for LFP recording immediately. Multiple analyses were performed in attempts to define localized responses. Isolating the frequency with the strongest Fourier response, Peak Response Frequency (PRF), indicated that stimulation frequency is broadly represented across stimulation locations and electrodes. Percent Voltage Response (PVR) shows localization of four locations and stimulation frequency-dependent response intensity. Finally, investigating Phase Lag Index (PLI) indicated interchannel desynchronization during increased voltage responses.

Analyses provided complementary insights into the cortical response to cutaneous vibrations on the face. PRF offered stimulation frequency identification, but at the cost of both electrode and stimulation site localization. PVR offered some intensity mapping but provided localization for channels and locations. PLI suggests insight into (de)synchronization that can use PRF's broad activity and PVR's localization to create a more comprehensive map.

Disclosures: J.C. Tanner: None. **T. Hearn:** None. **S. Helms Tillery:** None.

Poster

618. Receptive Field and Response Properties of Tactile Sensation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 618.02/PP1

Topic: D.03. Somatosensation: Touch

Support: NINDPS DP2NS087725-01

NEI R01EY023756-01

New York Stem Cell Foundation

Title: Complex feature coding in the somatosensory cortex through stimulus specific supra-linear integration

Authors: *S. PLUTA[1], H. ADESNIK[2];
[1]Mol. Cell Biol., Univ. of California Berkeley Dept. of Mol. and Cell Biol., Berkeley, CA;
[2]Univ. of California, Berkeley, Berkeley, CA

Abstract: Rodents explore their environment by actively palpating objects with a large array of facial vibrissa. However, due to technical limitations, how somatosensory cortical neurons encode complex multi-whisker interactions during active sensation is poorly understood. To address this question, we developed a novel multi-pneumatic actuator capable of testing arbitrary patterns of single and multi-whisker touch in awake, whisking mice. Contrary to research using anesthetized animals, we found that the majority of layer 5 pyramidal neurons display supra-linear multi-whisker integration during active sensation. Surprisingly, supra-linear integration primarily resulted from multi-whisker disinhibition rather than super-additive summation; when stimulated alone, adjacent whiskers typically drove suppression, but when stimulated in specific combinations with other nearby whiskers, adjacent whiskers were highly facilitating. In the minority of pyramidal neurons that lacked adjacent whisker suppression, linear or sublinear integration was observed. Therefore, higher order tactile features may be extracted during touch through a stimulus specific disinhibitory circuit. We are currently optogenetically deactivating descending cortical projection neurons to elucidate their role in surround suppression and nonlinear integration.

Disclosures: S. Pluta: None. **H. Adesnik:** None.

Poster

618. Receptive Field and Response Properties of Tactile Sensation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 618.03/PP2

Topic: D.03. Somatosensation: Touch

Support: DARPA HAPTIX N66001-15-C-4018

Title: Encoding touch perception for an intrafascicular peripheral nerve interface

Authors: E. PATRICK[1], A. KUNDU[1], K. OTTO[1], J. PRINCIPE[1], R. BASHIRULLAH[1], *A. GUNDUZ[2];
[2]Biomed. Engin., [1]Univ. of Florida, Gainesville, FL

Abstract: Somatosensory feedback is necessary to achieve highly functional peripheral neuroprosthetics. The development of neural interfaces that are capable of peripheral nerve stimulation to provide the missing sensory information is a topic of current research. The challenge facing all interface technologies is to provide stimulation patterns that will induce realistic sensory percepts with high spatial sensitivity. The Implantable Multimodal Peripheral REcording and Stimulation System (IMPRESS) is a novel interface designed for highly specific intrafascicular recording and stimulation due to its high channel count and electrode density. This work provides fundamental information for development of an encoding strategy to be used in the IMPRESS interface.
A combination of rate and population encoding is anticipated to be used in our biomimetic approach. The design constraints that need to be understood are then 1) the spatial extent of axon recruitment for a given stimulus and 2) the endogenous firing patterns corresponding to different mechanoreceptors that encode tactile pressure intensity. Our experimental procedure consists of tactile stimulation of the glabrous skin of the rat hind paw and subsequent recording via microelectrodes placed in the main fascicle of the sural nerve and the L5 dorsal root ganglion (DRG). The efficacy of encoding stimulation patterns are tested by providing stimulation through the intrafascicular sural nerve electrodes and recording from the DRG-implanted electrodes. A computational model for prediction of recruitment of individual fibers is validated and tuned by the results of this experimental work.

Disclosures: **E. Patrick:** None. **A. Kundu:** None. **K. Otto:** None. **J. Principe:** None. **R. Bashirullah:** None. **A. Gunduz:** None.

Poster

618. Receptive Field and Response Properties of Tactile Sensation

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Program#/Poster#: 618.04/PP3

Topic: D.03. Somatosensation: Touch

Support: CIHR MT-5877

NINDS NS090595

NINDS NS058668

NSF PolS 2014906

Title: Subnucleus muralis: first relay for sensory input from the glabrous orofacial skin

Authors: *A. CALLADO PÉREZ[1], D. KLEINFELD[2], M. DESCHÊNES[1];
[1]Univ. Laval, Quebec, QC, Canada; [2]UCSD, San Diego, CA

Abstract: Quadrupeds frequently investigate objects of interest and congeners with their narial pads. Yet, few studies have examined the sensory physiology of these ethologically important organs. Here, we show that injection of cholera toxin beta subunit into the narial pad leads to terminal labeling in the transition zone between the interpolaris and caudalis subnuclei of the trigeminal sensory complex, i.e. subnucleus muralis. We also provide physiological evidence that muralis cells respond to mechanical stimulation of the narial pad or the nasal and buccal mucosae. Some muralis cells are also activated by inhalation of ammonia vapor. Response latencies indicate that these sensory inputs are conveyed by Aδ and C-fibers. Yet, none of the muralis cells responded to vibrissa deflection. Sindbis-GFP injection into subnucleus muralis leads to anterograde labeling in the facial nucleus and the superior salivatory nucleus. Projection to the facial nucleus was also confirmed by transsynaptic labeling after pseudorabies injection into the superior lip. Together with prior studies that demonstrated that subnucleus muralis receives input from the cornea, our results suggest that this trigeminal subnucleus might process sensory inputs from the glabrous skin of the face : the narial pads, the lips and external epithelium of the eyes.

Disclosures: A. CALLADO Pérez: None. **D. Kleinfeld:** None. **M. Deschênes:** None.

Poster

618. Receptive Field and Response Properties of Tactile Sensation

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Topic: D.03. Somatosensation: Touch

Support: PROTOTOUCH EU FP7 project 317100

Swedish Research Council grant 3548

Vastra Gotaland Regional Council ALFGBG-441901

Title: Tactile afferent responses to mechanical pink noise bursts of varying amplitude and duration in humans.

Authors: *M. AMANTE[1], R. WATKINS[1], S. BOCHEREAU[2], R. ACKERLEY[1], J. WESSBERG[1], V. HAYWARD[2];
[1]Physiol., Univ. of Gothenburg, Göteborg, Sweden; [2]UPMC Paris, Paris, France

Abstract: In vision and audition, the perceived intensity of a short stimulus is dependent on its duration. The same phenomenon was recently reported for the sense of touch in a psychophysical study (1). Bursts of mechanical pink noise was chosen to mimic the broadband vibration characteristics of textures and surfaces encountered in daily life. Similar to the other senses, it was found that shorter bursts were consistently perceived as weaker than bursts of the same intensity but longer duration. Hence, there is perceptual duration-intensity constancy for tactile stimuli of short duration. The aim of the present study was to explore to what extent this reflects coding of intensity in peripheral tactile receptors. We used the microneurographic technique to record the responses of single, identified tactile receptor units in the median nerve in healthy volunteers. We constructed a stimulator probe with a speaker coil motor mounted on a one-axis actuator with acceleration and force sensors. The motor was driven by pink noise bursts with varying intensity and duration (100 - 700 ms; 2-40 m/s^2). Interstimulus intervals were 300 - 800 ms. The aim was to produce controlled skin vibrations rather than a sliding stimulus; hence, the rounded tip (4 mm diam.) of the probe was covered with fine grit sandpaper (P600) to achieve high friction. Single afferent units were classified as SA1, SA2, FA1 or FA2, and the receptive field of each unit was mapped using von Frey hairs. The probe was positioned to contact the receptive field of the single unit using normal (90 degrees angle to the skin) and tangential probe orientations, and the normal contact force was kept at 40 mN under closed-loop actuator control. The tangential probe orientation consistently elicited weaker responses compared to a normal orientation. Increasing the duration and/or amplitude of the stimulus predictably resulted in increased receptor responses in all classes of afferents, allowing for estimation of the interdependence of amplitude and duration in the population of activated afferents. However,

coding of stimulus intensity can in theory rely on several factors, or their combination: i) Recruitment and single nerve discharges in tactile receptors that are stimulated near their mechanical thresholds; ii) increased number of elicited nerve discharges in the recruited receptors; iii) increased average or peak firing rates. Hence, there is a putative role for afferents that are activated both at near- and supra-threshold intensity in the coding of intensity in the tactile system.

1. Bochereau S, Terekhov A & Hayward V. Lecture Notes in Computer Science, 2014, 8618, 93-100

Disclosures: **M. Amante:** A. Employment/Salary (full or part-time): Göteborg universitiet. **R. Watkins:** None. **S. Bochereau:** None. **R. Ackerley:** None. **J. Wessberg:** None. **V. Hayward:** None.

Poster

618. Receptive Field and Response Properties of Tactile Sensation

Location: Halls B-H

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Program#/Poster#: 618.06/PP5

Topic: D.03. Somatosensation: Touch

Support: NINDS R01NS073119 EAL GJG

Title: Diversity of rapidly adapting and slowly adapting responses in mouse hairy skin

Authors: ***Y. BABA**, E. A. LUMPKIN;
Dermatol., Columbia Univ. Med. Ctr., New York, NY

Abstract: Cutaneous mechanosensory neurons can be classified by physiological characteristics, including threshold (low and high), conduction velocity (Aβ, Aδ, and C), and adaptation (rapidly adapting: RA and slowly adapting: SA). Recent studies have begun to establish the relationship between physiological characteristics and receptive field structures in mouse hairy skin. For example, neurons innervating guard hairs (GH) are reported to be Aβ RA low-threshold mechanoreceptors (LTMRs), whereas neurons innervating touch domes (TD) are thought to be Aβ SAI LTMRs. Using an ex vivo skin-nerve preparation, we surveyed the relationship between Aβ and Aδ afferents and receptive fields. Surprisingly, more than half of the GH-associated afferents produced SA responses. Most TD neurons were Aβ SAs; however, we observed Aβ SA afferents that did not respond to TD stimulation. About 70% of afferents did not contact GHs or TDs. This group included D-hair afferents, which were very sensitive LTMR neurons (median von Frey threshold: 0.2 mN) that responded to movement of zigzag hairs. Most showed low Aβ and Aδ conduction velocities and RA responses. About half of this sub-group showed SA

responses to strong stimulation. Other SA afferents were classified into two groups; high-threshold afferents, akin to A mechanonociceptors (AM), and field LTMRs. Both types responded to skin indentation rather than zigzag hair movement. The former had relatively high thresholds, and conduction velocity ranged low Aβ and Aδ. Field LTMRs displayed low thresholds and conduction velocities in the Aβ and fast Aδ. Finally, we observed a group of high-threshold RA afferents that did not respond to zigzag hair stimulation or punctate stimuli, but instead to wide-field compressive stimuli. These data show that at least three types of RA neurons (GH-RA, zigzag-RA/D-hair, and wide-high-threshold-RA) and six types of SA neurons (TD-SA, TD-GH-SA, GH-SA, zigzag-SA/D-Hair, field-LTMRs, and high threshold skin-indentation-SA which is conventionally named AM). This classification scheme can account for about 90% of Aβ and Aδ LTMRs in the hairy skin of the proximal hind-leg in C57BL/6J mice.

Disclosures: Y. Baba: None. **E.A. lumpkin:** None.

Poster

618. Receptive Field and Response Properties of Tactile Sensation

Location: Halls B-H

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Program#/Poster#: 618.07/PP6

Topic: D.03. Somatosensation: Touch

Support: Samsung Research Funding Center of Samsung Electronics Grant SRFC-IT-1502-05

Title: Optimizing ultrasound parameters for tactile sensation

Authors: *J. B. CHOI[1], K. W. CHO[1], M. K. SONG[1], M.-K. JEONG[2], S. I. KIM[1], I. Y. KIM[1], D. P. JANG[1];
[1]Hanyang Univ., Seoul, Korea, Republic of; [2]Daejin university, Kyeonggi, Korea, Republic of

Abstract: There are various receptor types or free nerve endings underneath skin. Human tactile sensation is basically a combination of these receptors, and each receptors have own characteristics for mechanical, vibrational stimulation on skin surface. Recently, focused ultrasound have been reported as a new tool for evoking tactile sensation in skin. The Russian researcher, L. R. Gavlirov, first reported the effect of focused ultrasound stimulation on human hand, and found that focused ultrasound stimulation evoked various skin sensations, but its underlying mechanism was unknown. Another study performed by researchers in University of Bristol found that modulating the pulse frequency of focused ultrasound stimulation induces receptor specific tactile sensations in human hand, but the underlying mechanism of focused ultrasound stimulation in skin tissue remains unknown yet. The main reason of unknown mechanism is lack of reliability in experiment result since these studies have to explain focused

ultrasound stimulation induced tactile sensations based on subjective explanation of human subjects. The tactile sensations induced by focused ultrasound stimulation are not a kind of 1common feelings in everyday life, most of subject have difficulty with explaining the tactile sensations induced by focused ultrasound stimulation in their hands. In this study, therefore, we aim to find optimized ultrasound parameters for stimulating mechanoreceptors in skin, based on subject's experience and neural response from animal. Recent result shows that the amount of radiation force which includes pressure level, impedance difference, and attenuation ratio could be the prime parameters of focused ultrasound stimulation, but further experiment would be required. Configuring the database from the experiment result of human subject's report and spiking patterns of animal subjects associated with stimulation parameters (frequency, duration, and so on), could be the basic principles and resources for the development of tactile display in the future.

Disclosures: J.B. Choi: None. **K.W. Cho:** None. **M.K. Song:** None. **M. Jeong:** None. **S.I. Kim:** None. **I.Y. Kim:** None. **D.P. Jang:** None.

Poster

618. Receptive Field and Response Properties of Tactile Sensation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 618.08/PP7

Topic: D.03. Somatosensation: Touch

Support: DAAD

Title: Voltage sensitive dye imaging of leech local bend network

Authors: *E. FATHIAZAR, S. MEISER, A. TRENDE, J. KRETZBERG;
Dept. for Neurosci., Univ. of Oldenburg, Oldenburg, Germany

Abstract: Touch location discrimination on leech body wall is as precise as the human fingertip, while the nervous system of leech is rather small. Each leech mid-body ganglion contains 400 individually characterized neurons and is able to control local reflexes entirely from sensory inputs, via interneuron computation, resulting in motor neuron activity and corresponding behavior. In the local bend reflex the leech locally bends away the body wall from the location of a light tactile stimulus. While the encoding of touch stimuli was studied in terms of spiking attributes of sensory cells [1], the principles of sensory information processing on the level of interneurons remains to be investigated.
Here we used voltage sensitive dye (VSD) imaging to reveal the network of neurons involved in local bend reflex. A series of signal processing steps, e.g. movement artifact removal and moving

average, were applied to the raw VSD signal to overcome the noise and artifacts. A simultaneous intracellular recording of one cell was used to relate the VSD signal to the real membrane potential and to test the effect of this cell'. We performed these recordings in a semi-intact skin preparation with the ganglion attached to a patch of skin, which was stimulated with a mechanical poker to elicit the local bend response [1].
VSD signals of up to 100 simultaneously recorded neurons located on the ventral surface of the ganglion were analyzed with a statistical classification approach. More than half of the visible cells were classified as stimulus-activated, showing significantly different activity between with and without skin stimulation. In addition to the mechanosensory cells, in particular the so-called AP cell was found as one of the stimulus-activated cells consistently between preparations. Double intracellular recordings and anatomical studies revealed that the AP cell receives inputs from all mechanosensory pressure (P) cells with stronger contralateral connections.
The next analysis step aims at identifying network topology and causal relations between different cell types. Since leech interneuron spikes measured in the soma are small (usually < 5 mV), the computation of sensory information is probably based on the considerable graded membrane potential changes occurring during tactile stimulation. We compare the performances of different time series analysis techniques (Granger causality, convergent cross mapping, information theoretic approaches) applied to these small and noisy signals for revealing network structures matching physiological knowledge and identifying stimulus-dependent network states.
[1] Pirschel F, Kretzberg J, *J Neurosci* 2016, 36(13):3636–3647

Disclosures: **E. Fathiazar:** None. **S. Meiser:** None. **A. Trende:** None. **J. Kretzberg:** None.

Poster

618. Receptive Field and Response Properties of Tactile Sensation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 618.09/PP8

Topic: D.03. Somatosensation: Touch

Support: PROTOTOUCH EU FP7 project 317100

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Västra Götaland Regional Council ALFGBG-441901

Title: Afferent responses to tactile stimulation by natural textures during active touch in humans

Authors: ***M. DIONE**, R. WATKINS, R. ACKERLEY, J. WESSBERG;
Univ. of Gothenburg, Goeteborg, Sweden

Abstract: Significant advances have been made in the last 20 years to provide amputee individuals with limb prostheses enabling the execution of movements. In the close future, one can expect that prostheses will also supply relevant tactile feedback back to the nerves for the restoration of natural touch sensations after limb amputation. However, it is still unclear how afferent information is conveyed from the skin to the brain during natural active touch to elicit specific tactile percepts in healthy individuals. In the present study, we used microneurography to record from Aβ-mechanoreceptive afferents in awake human subjects. We studied afferent responses when the skin was stimulated by subjects actively stroking across natural textures. During active touch, specific skin dynamics are reflected as global variations in the lateral force. During a lateral stroke, there is an initial increase in lateral force, a phase of stable sliding and a final decrease in lateral force. This specific force pattern could elicit a specific firing pattern in the different mechanoreceptor classes. In humans, the skin also stretches during active touch, and this could provide a relevant code to characterize the level of friction exerted by textures. In the present study, afferent responses were recorded from the left median nerve (7 FA1, 4 FA2, 4 SA1, 7 SA2) while participants were asked to produce lateral stroking movements of the index or middle finger over a range of natural textures, using comfortable force and preferred slow or fast speeds. Finger position, acceleration, EMG, and the forces applied on the textures were also recorded. For all units, at least one texture was tested in most of the conditions. Our results confirmed that the different classes of afferent unit have specific signature that relate to the global changes in lateral force occurring during active touch. SA2 units responded more at movement initiation and termination, SA1 at sliding initiation, FA2 and FA1 during sliding. In addition, for 1 FA1, 1 FA2 and 1 SA2 a larger number of textures was tested (n= 15 to 18). For these units, we computed the correlations between the mean firing rate and the friction coefficient. Correlations were significant in all units but were positive for the SA2 and negative in the FA units. We conclude that the mechanoreceptor classes respond differentially to the dynamic motor events occurring during to active touch and that the level of skin stretch coded by SA2 units is relevant to produce tactile coding of natural textures in humans. We hope human microneurography data will inspire research on sensory feedback to restore natural sensations in limb injured individuals with nerve-wired bionic limbs.

Disclosures: **M. Dione:** None. **R. Watkins:** None. **R. Ackerley:** None. **J. Wessberg:** None.

Poster

618. Receptive Field and Response Properties of Tactile Sensation

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Topic: D.03. Somatosensation: Touch

Support: CAD NIH Grant# MH100749

CAD; MGR; CIM: NIH Grant# NS045130 (to CIM)

Title: Top-down driven functional heterogeneity among inhibitory neurons improves tactile representation and detection

Authors: *C. A. DEISTER[1], M. GOMEZ-RAMIREZ[1], J. VOIGTS[2], C. I. MOORE[1];
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Abstract: Neurons in sensory cortices show functional dynamics in evoked rates and patterns. These dynamics may shape perceptual behaviors by improving the detectability or discriminability of ambiguous stimuli. Feedforward and feedback interactions among cortical areas drive these dynamics, but we do not have a complete cellular/circuit level understanding of how. Recent work provides correlative and causal links between receptive field properties and the activity of feedback projections in the performance of sensory detection tasks. But, if and how these two phenomena are linked are not known.
To address this, we trained mice to perform a vibrissa-motion detection task. Mice become more sensitive to weak stimuli after many days of training. We imaged populations of GCaMP6f expressing pyramidal neurons and parvalbumin-positive (PV+) interneurons in layers 2/3 and 5a of vibrissa somatosensory cortex (vS1; barrel cortex). Putative ensembles of pyramidal and PV+ neurons showed evoked rate and correlation changes that predicted successful detection. While these neuron's responses were specifically correlated with task performance, the majority of these neurons were generally responsive to the same vibrissa deflections before training. In contrast, when we imaged the activity of S2->S1 projections or local somatostatin-containing (SST+) interneurons, we found weak trial-to-trial correlations or rate changes evoked by weak deflections before training. Significant correlations and reliable and predictive evoked rate changes emerged among many (~40%) SST+ neurons. The activity of S2->S1 projections showed similar task-related changes. Imaging identified SST+ alongside pyramidal neurons showed that 'task-recruited' SST+ neurons were positively correlated with predictive pyramidal neurons, but only on perceived trials. Simple, but plausible, network models were made from conductance-based integrate and fire neurons in order to make a circuit level hypotheses informed by our imaging data. A simple model in which top-down input creates transient 'imbalances' in classical feedforward sensory-driven excitatory and inhibitory coupling explained our data well. Our results suggest that the sparse nature of the cortico-cortical layers of vS1 and general cortical interneuronal diversity can be exploited by top-down inputs to create or reinforce conjoint representations between incoming thalamic sensory relay and ongoing cortico-cortical activity. We are currently employing discrimination tasks in order to help determine if the described dynamics are a general mechanism for emphasizing specific, but distinct, sensory features.

Disclosures: **C.A. Deister:** None. **M. Gomez-Ramirez:** None. **J. Voigts:** None. **C.I. Moore:** None.

Poster

618. Receptive Field and Response Properties of Tactile Sensation

Location: Halls B-H

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Program#/Poster#: 618.11/PP10

Topic: D.03. Somatosensation: Touch

Support: NIDCD Grant DC-00046

Title: Hidden sources of variability modulate populations of sensory neurons

Authors: *M. WHITEWAY[1], D. A. BUTTS[2];
[1]Applied Mathematics, [2]Biol., Univ. of Maryland, College Park, MD

Abstract: Sensory cortex not only represents information from sensory receptors, but also incorporates other sources of input from recurrent and top-down connections. Unlike sensory stimuli, these other sources of input are difficult or impossible to control experimentally, and as a result contribute to neuronal variability in most experimental contexts. However, such sources of variability are likely to play an integral role in sensory cortex function. We introduce a new computational model, based on a type of neural network called an autoencoder, to identify these sources of variability. We first show that the autoencoder performs better than other common "latent variable" models including principal component analysis, independent component analysis, and factor analysis, across a variety of measures using simulated data. We then apply this model to a publicly available dataset (Peron et al. Neuron 2015), where large populations of neurons in mouse barrel cortex are recorded during a decision-making task using two-photon imaging. Across many experiments this method robustly identifies a small number of variables contributing to the cortical population activity. Some of these variables are correlated with the stimulus, while others are correlated with non-stimulus trial variables. These results suggest the activity of a large proportion of neurons in primary sensory cortex is modulated by hidden sources of variability that are not directly related to the stimulus. Identifying such sources of variability thus sets the foundation for understanding the role of such variables in sensory cortical function.

Disclosures: M. Whiteway: None. **D.A. Butts:** None.

Poster

618. Receptive Field and Response Properties of Tactile Sensation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 618.12/PP11

Topic: D.03. Somatosensation: Touch

Title: Tactile spatial acuity of the neck using a two-point orientation discrimination task

Authors: K. MORROW, *M. ZIAT;
Psychology, Northern Michigan Univ., Marquette, MI

Abstract: This study explores the tactile acuity of the neck using a two-point orientation discrimination (2POD) task with 16 human participants (10 female, M= 19.53, SD= 1.16). A modified drafting compass was used to deliver pressure stimuli in two orientations (vertical and horizontal), and participants were asked to discriminate the orientation of the two compass points at eight locations on the neck. Using a two-up, one-down staircase method, thresholds were found for each site. A one-way repeated measures ANOVA shows a significant effect of the factor location [$F(7, 70) = 4$, $p= 0.01$] . Post-hoc analysis shows that the very front of the neck is slightly more sensitive than other locations. Additionally, all the other tested sites presented uniform spatial tactile acuity with an average threshold of 13.75 mm. The uniform spatial resolution of the neck can be advantageous for perceiving directional information used for potential clinical applications such as sensory substitution or brain-computer interfaces.

Disclosures: K. Morrow: None. **M. Ziat:** None.

Poster

618. Receptive Field and Response Properties of Tactile Sensation

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Program#/Poster#: 618.13/PP12

Topic: D.03. Somatosensation: Touch

Title: Sensorimotor and tactile discrimination deficits in barrel cortex following Alzheimer's disease: the role of nucleus basalis of Meynert in somatosensory information processing

Authors: *B. SADEGHI[1,2];
[1]The Neurosci. Res. Ctr., Shahid Beheshti Univ. of Med. Sci., Tehran, Iran, Islamic Republic of;
[2]Intl. Inst. of Biochem. and Biophysics (IBB), Univ. of Tehran, Tehran, Iran, Islamic Republic of

Abstract: Integration of neuronal responses across multiple whiskers is crucial for an effective facial vibrissae somatosensation. In memory processing, the main origin of the cholinergic projection to the neocortex appears to be the magnocellular neurons in the region of the nucleus basalis of Meynert (NbM). Here, in order to identify the prominent mechanisms underlying the modulation of the somatosensory information processing in cases with Alzheimer's disease (AD) as a cognitive degenerative disorder, the effects of chemical lesions to the nbM on the temporal characteristics of response integration evoked by multiple whisker stimulations were investigated in the barrel cortex of male Wistar rats. Animals were randomly distributed into three groups; control group, sham operated and rats that received bilateral excitotoxic lesion (by ibotenic acid in Alzheimer's group). In order to assess behavioral and cognitive functions via the determination of characteristics of excitatory and inhibitory receptive fields (RFs), passive avoidance learning (PAL) task was done and extracellular single-unit recordings from layer V barrel cortex neurons of anesthetized rats were performed following the single or paired, rostral and caudal deflection of two neighboring principle and adjacent whiskers (PW, AW) in the same row at varying interstimulus intervals (ISIs). Results show that nbM lesion significantly decreased response magnitude but did not affect response latencies. Response onset latency to both PW, AW in ibotenic acid (IBO)-injected animals were not significant compared with the control group; but unlike the AW, the magnitude of ON response to PW deflection in IBO-injected animal was significantly lower (nearly 20%) than control group. Unit's discharges to subsequent deflection of AW were reduced. The magnitude of responses were significantly reduced at each inter-deflection interval in nbM lesioned group. The excitatory RFs of units were extended in nbM lesioned animals and nbM lesion increased surround inhibition. Results from the PAL task reveal that step-through latency was decreased in rats with AD which gives an indication of memory loss. It has been suggested that interactions of nearby whiskers and the resulting facilitation and inhibition in the barrel cortical neurons have an important role in translating discrete information from individual whiskers to a continuous space. Thus, the nbM has an important role in regulating the balance of excitation and inhibition in the barrel cortex and can influence the temporal integration of tactile inputs and stimulus perception in AD by modulating the sensory information processing.

Disclosures: B. Sadeghi: None.

Poster

618. Receptive Field and Response Properties of Tactile Sensation

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Program#/Poster#: 618.14/PP13

Topic: D.03. Somatosensation: Touch

Support: Barkley Trust Foundation (Barlow)

Title: Brain encoding of stimulus velocity within a saltatory pneumotactile array in the human perioral somatosensory system using fMRI

Authors: *R. CUSTEAD[1], H. OH[2], Y. WANG[2], S. M. BARLOW[3];
[1]Communication Disorders, Neurosci., Univ. of Nebraska at Omaha Sch. of Communication, Lincoln, NE; [2]Biol. Systems Engineering, Ctr. for Brain, Biol. & Behavior, [3]Communication Disorders, Biol. Systems Engineering, Ctr. for Brain, Biol. & Behavior, Univ. of Nebraska-Lincoln, Lincoln, NE

Abstract: BACKGROUND: Processing dynamic tactile inputs is a key function of somatosensory systems. Spatial velocity encoding mechanisms by the nervous system are crucial for skilled movement production and recovery of motor function following neurological insult. Little is known about tactile velocity encoding in trigeminal networks associated with external sensory inputs to the face, or the somatosensory consequences of movement. OBJECTIVE: To use high resolution functional magnetic resonance imaging (fMRI) to investigate neural substrates of velocity encoding in the human orofacial somatosensory system during saltatory, pneumotactile inputs to the unilateral orofacial skin. METHODS: Participants - 20 neurotypical, right-handed adults, age 19-30 years. A multichannel, scalable pneumotactile array (Galileo Somatosensory) consisting of 7 TAC-Cells was used to present 5 stimulus conditions: 5 cm/s, 25 cm/s, 65 cm/s, ALL-ON synchronous activation, and ALL-OFF, to the hairy skin of the right perioral lower face. A T1-weighted MPRAGE sequence (0.9mm isotropic, TE=3.37ms, TR=2400ms) was followed by 3 functional scans using a 3T Siemens Skyra (32-ch head coil). Functional images: T2*-weighted EPI sequence, 41 slices (2.5x2.5x2.5mm, TE=30ms, TR=2.5s, FOV=220mm). Using SPM12, acquired brain volumes/subject were realigned, and smoothed with an isotropic Gaussian kernel (FWHM=8 mm). The spatial organization of cerebral and cerebellar blood oxygen level-dependent (BOLD) response as a function of stimulus velocity was analyzed using general linear modeling (GLM) of pooled group fMRI BOLD data. RESULTS: Sequential saltatory inputs to the lower face produced localized, predominantly contralateral BOLD responses in primary somatosensory (SI), secondary somatosensory (SII), primary motor (MI), supplemental motor area (SMA), posterior parietal cortices (PPC), and insula, whose spatial organization was highly dependent on velocity. Additionally, ipsilateral sensorimotor, insular and cerebellar BOLD responses were prominent during the lowest velocity

presentation (5 cm/s). CONCLUSIONS: Results indicate significant modulation in the spatial extent and region of BOLD signal as a function of stimulus velocity, reflecting a dynamic sensorimotor network underlying the processing of saltatory pneumotactile stimuli presented to the human trigeminal system. This regional modulation may be related to unique velocity processing and motor planning networks associated with tactile discrimination of movement across orofacial skin.

Disclosures: R. Custead: None. **H. Oh:** None. **Y. Wang:** None. **S.M. Barlow:** None.

Poster

618. Receptive Field and Response Properties of Tactile Sensation

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Program#/Poster#: 618.15/PP14

Topic: D.03. Somatosensation: Touch

Support: NSERC Discovery Grant

Title: Lower-limb cutaneous reflexes evoked via discrete and continuous mechanical foot sole stimulation

Authors: *R. M. PETERS, R. L. MILDREN, M. G. CARPENTER, J.-S. BLOUIN, T. INGLIS; Sch. of Kinesiology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Cutaneous reflexes are traditionally evoked using high-frequency electrical pulses delivered either to the whole nerve (Aniss *et al*., 1992) or to specific skin regions (Zehr *et al*., 2014). While this approach has uncovered interesting reflex properties (e.g., gait phase-dependent reflex reversal; Zehr *et al*., 2014), two disadvantages of electrical stimulation exist: 1) it non-specifically activates all mechanoreceptor classes, and 2) it entrains afferents to a non-physiological input (e.g., 300 Hz pulse train). Here we have taken the novel approach of using mechanical stimuli to elicit lower-limb reflexes, which rely on natural mechanotransduction, and given the frequency selectivity of plantar skin receptors (Bent and Strzalkowski, 2014), can bias the population response toward specific afferent classes. Subjects were 20 healthy young adults (mean age = 25.6, SD = 3.4). Stimuli consisted of either 600 *discrete* sinusoidal pulses (one cycle at 30 Hz) or 120 s of *continuous* stochastic vibration (bandwidths: 0 to 30 Hz and 0 to 300 Hz), delivered to the plantar skin overlying the right first metatarsal head at amplitudes of 3 and 10 times perceptual threshold (PT). Surface and indwelling EMG recordings were obtained from Tibialis Anterior while participants maintained a constant level of ankle dorsiflexion (12.5 and 25% of MVC). Responses were obtained by either stimulus-triggered EMG averaging (discrete), or by computing the coherence and cross-covariance between the stimulus acceleration/force and

EMG (continuous). Regardless of stimulus type, peak responses were observed at latencies (~90-100 ms) consistent with expectations of a cutaneous-mediated spinal reflex (Kavounoudias *et al*., 2001; Forth & Layne, 2007). Larger reflex responses were observed with higher-amplitude stimulation (10 vs. 3 PT), as well as with greater background EMG (25 vs. 12.5% MVC). For continuous stimuli, significant coherence was commonly observed only up to ~30 Hz. Given discrete and continuous methods provide similar reflex response estimates, and the advantages of continuous stimuli in terms of trial duration and the ability to probe a broad-range of frequencies, we argue that stochastic stimulation is a powerful new technique for studying cutaneous reflex function.

Research funded by NSERC

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Poster

618. Receptive Field and Response Properties of Tactile Sensation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 618.16/PP15

Topic: D.03. Somatosensation: Touch

Support: NSF IGERT DGE-1069104

UMN Interdisciplinary Doctoral Fellowship

Title: An information theory analysis of the response of the in silico Pacinian corpuscle to noisy and complex stimuli

Authors: *J. QUINDLEN, B. J. YOUNG-DIXON, E. T. BLOOM, V. H. BAROCAS;
Biomed. Engin., Univ. of Minnesota, Minneapolis, MN

Abstract: The Pacinian corpuscle (PC) is a cutaneous mechanoreceptor that responds to 20-1000 Hz vibrations, which are transmitted through the PC's end organ to its central nerve fiber

(neurite). We previously developed a mechanical and neural model of PC mechanotransduction. The model outputs neural activity resulting from a vibration applied to the PC's outer surface. The model accounts for transmission of vibration through the PC's outer core, subsequent strain on the neurite, opening of mechanogated channels, and generation of action potentials. The neural signal in response to vibration at different frequencies and amplitudes was simulated for 10 Hz - 1kHz vibrations. Simulations resulted in variable firing rates (FRs) at low amplitudes followed by a static region at higher amplitudes, at which the FR was equal to the indentation frequency.
The purpose of the current study was to draw insight into the transmission of information by the PC in response to vibration and observe the effect that a noisy mechanical input (e.g., due to noise in the stimulus itself or inaccuracy arising from transmission through the skin) has on the signal's mutual information. The mutual information of neurite firing resulting from stimulation at different frequencies was calculated in noise-free and noisy vibrations with two different output parameters: FR and interspike intervals (ISIs). Vibrations of 75, 100, 150, and 160 Hz were chosen for analysis because they can have either overlapping outputs (i.e. same FR) or distinct outputs (i.e. different FR) based on the stimulation amplitude. Gaussian white noise was added to the input to simulate a noisy vibration. FRs and ISIs of the generated neural signals were measured and mutual information was calculated to observe the rate and temporal information transmitted by the signal. The mutual information calculated from the ISI of the noise-free signals was 0.96 bits, which decreased to 0.90 bits for low noise (1 to 20 dB SNR), 0.81 bits for medium noise (-7 to 13 dB), and 0.73 bits for high noise (-19 to 1 dB). The rate codes showed no mutual information change for all chosen noise values (-19 to 20 dB SNR). Thus, the presence of noise has a greater effect on the temporal coding of the PC's neural signal. The next step is to quantify the information contained in a complex (multi-frequency) mechanical vibration. The effect of variables such as the base frequency, dissonance, and phase difference between waveform components will be investigated to draw parallels to published psychophysical experiments. The overall aim is to quantify the information content transmitted in response to simple and complex vibrations and to observe the effect of noise on this value.

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Poster

618. Receptive Field and Response Properties of Tactile Sensation

Location: Halls B-H

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: DARPA HR0011-15-2-0054

Title: Peripheral nerve stimulation in mice via non-invasive focused ultrasound

Authors: ***M. DOWNS**, G. Z. X. YANG, Q. WANG, E. E. KONOFAGOU;
Biomed. Engin., Columbia Univ., New York, NY

Abstract: Background. The use of focused ultrasound (FUS) to non-invasively modulate neurons has recently gained interest as a potential technique to treat neurological disorders. It has been shown to have both a stimulatory and inhibitory effect when applied to ex vivo peripheral nerves, mainly harvested from the crab and frogs, with one study stimulating, and recording from the vagus nerve in vivo with rats. Thus far, there have been no studies determining if stimulating peripheral nerves with FUS can have physiological effects with an in vivo model. Research Questions. Two questions regarding the use FUS as a technique to stimulate peripheral nerves are addressed herein: 1) Can FUS be used to stimulate peripheral nerves in the mouse model and elicit a physiological response (muscle activation)? 2) Can the FUS nerve stimulation be suppressed by administering an activation inhibitor to the targeted region? Results. Focused ultrasound was used to target the saphenous nerve in mice while recording EMG responses from the tibialis anterior muscle. Voltage (0.1-0.9V), duty cycle (1-90%) and duration (4ms-1s) were varied to determine threshold parameters for safe and efficient stimulation. 8 anesthetized mice had both hind limbs stimulated for a total of n = 16 limbs. Safe (no detectable tissue damage or morphological changes via H&E) EMG and muscle movement responses were observed for parameters up to 0.9 V at a 90% duty cycle for 10 ms FUS stimulation duration. There was an average delay of 25.4 ms between FUS stimulation and EMG response. Reduction of the output FUS intensity and duty cycle, but extending the duration did not elicit EMG responses. EMG responses increased linearly with the output intensity while maintaining a 90% duty cycle with a 4-9ms FUS stimulation duration. EMG response were significantly reduced during FUS stimulation 10 minutes after administering 0.5% lidocaine to the area targeted on the saphenous nerve. Conclusion. The findings of this study demonstrate that we can safely stimulate the saphenous nerve in mice and elicit muscle activation. This muscle activation was inhibited after application of a lidocaine nerve block to the targeted region of the saphenous nerve demonstrating that the nerve was stimulated by FUS. These findings demonstrate feasibility of ultrasound-induced peripheral nerve stimulation that provides opportunities for noninvasive stimulation of deep-seated nerves.

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Poster

619. Olfactory Circuits and Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 619.01/QQ1

Topic: D.04. Olfaction and Taste

Support: NIH grant R01013080

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Title: Modulation of olfactory-driven behavior by metabolic signals: Role of the piriform cortex

Authors: *D. AL KOBORSSY[1], V. CANOVA[2], M. THEVENET[2], S. GARCIA[2], B. PALOUZIER-PAULIGNAN[2], D. A. FADOOL[1], A. K. JULLIARD[2];
[1]Dept. of Biol. Sciences, Program in Neurosci., Florida State Univ., Tallahassee, FL; [2]Team "Olfaction: From Coding to Memory," Lyon Neurosci. Center, INSERM U1028-CNRS, Univ. Lyon 1, Lyon, France

Abstract: Olfaction is one of the major sensory modalities that regulates food consumption and is in turn regulated by the feeding state. Olfactory sensitivity is increased by fasting, decreased after satiety, and is perturbed by metabolic disorders and weight gain. These accumulated data strongly suggest that the olfactory bulb (OB) subserves as a metabolic sensor, and herein, we explore the role of further processing areas to similarly detect metabolic cues. Because mitral cells of the OB are glucose and insulin sensitive under the dependence of the Kv1.3 ion channel, we explored the presence of targeted receptors in the piriform cortex (PC) of Wistar rat. Using immunocytochemical approaches we now demonstrate that Kv1.3 labeling is localized to the lateral olfactory tract (LOT) and in layers 2 and 3 of the PC. Glucose transporter type 4 (GLUT4) and insulin receptor kinase (IR) were detected in the LOT, and PC layers 1, 2, and 3. In brain slices of the PC, we current-clamped principal cells in the whole-cell configuration to explore changes in excitability to glucose and insulin. 61% of sampled pyramidal cells (n=13) lost spike adaptation and significantly decreased initial instantaneous frequency (IF) (47.2 +/- 1.6 Hz control vs. 25.0 +/- 1.4 Hz low glucose) in response to a bath concentration change from 5 to 1 mM glucose. All sampled pyramidal (n=3) and semi-lunar cells (n=3) decreased initial IF by 28% and 33%, respectively, within 10 minutes of applying insulin (172 nM) to the bath. The spike firing frequency of 77% of sampled pyramidal cells (n=13) decreased (10.5 +/- 4 Hz control vs. 6.7+/- 4.3 Hz low glucose) in response to bath applied low glucose. Lastly, we tested

whether bilateral infusion of metabolic signals into the anterior PC could alter olfactory processing. Cannulated fasted rats received either insulin (172 nM), glucose (10 mM), margatoxin (a Kv1.3 peptide blocker, at 0.1 nM) or vehicle microinjections, and were immediately tested for olfactory discrimination using a habituation/dishabituation paradigm in conjunction with measured sniffing frequency inside a customized plethysmograph chamber. Using a cohort of 20 rats, all three molecules decreased olfactory discrimination and modified response onset and sniff frequency, with the most pronounced effect being attributed to insulin. These data suggest that metabolic signals can modulate complex olfactory behavior by acting directly on the PC; the higher cortical region receiving communication from the OB. Our working hypothesis concerning metabolic processing and neuromodulation attributed to energy homeostatic state, must be expanded to incorporate that of additional central olfactory targets.

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Poster

619. Olfactory Circuits and Behavior

Location: Halls B-H

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Program#/Poster#: 619.02/QQ2

Topic: D.04. Olfaction and Taste

Support: NIH Grant MH101293

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Title: Long-lasting enhancement of odor-evoked periglomerular activity after olfactory fear conditioning

Authors: *M. D. KASS, J. P. MCGANN;
Psychology, Rutgers Univ., Piscataway, NJ

Abstract: Olfactory fear conditioning can selectively alter the neural representations of odor stimuli as early as the primary sensory input to the brain (Kass et al 2013). This plasticity could be related to learning-induced changes in olfactory bulb glomerular circuitry, which controls sensory input gain and is dynamically regulated by neuromodulatory and cortical networks involved in fear learning. Here, we performed *in vivo* cell type-specific optical neurophysiology to evaluate the effects of olfactory fear conditioning on periglomerular (PG) circuitry in adult mice. Fear conditioning consisted of a single day of training with 10 trials of a ~15 sec odor (the

CS^+) paired with a footshock (or 10 shock or odor alone trials for control groups). We compared odor-evoked GCaMP signals in GAD65-expressing PG cells 1 day before, 1 day after, and 1 month after training in each individual mouse, and measured odor-evoked freezing in a novel context at similar post-training time points. During imaging and test sessions, subjects were presented with a panel of 4 odors including the CS^+ and 3 unexposed odors. Fear conditioned mice exhibited stimulus-evoked freezing that generalized across all 4 odors (after non-discriminative conditioning) and that was observed even 1 month after learning, whereas little freezing was observed in control animals. In parallel, we found that fear conditioning resulted in a robust, non-specific enhancement of odor-evoked GCaMP signals in GAD65-expressing PG cells. This generalized enhancement occurred just 1 day after learning and persisted up to 1 month. These data show that fear conditioning causes relatively rapid and long-lasting changes in olfactory coding. Such changes might decrease discrimination between threat-predictive and neutral stimuli and promote generalized anxiety.

Disclosures: M.D. Kass: None. **J.P. McGann:** None.

Poster

619. Olfactory Circuits and Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 619.03/QQ3

Topic: D.04. Olfaction and Taste

Support: NIH Grant DC011184

Title: Prenatal and early postnatal exposure to odorized food changes response properties of mitral cells in the mammalian olfactory bulb

Authors: *A. LIU[1,4,2,3], N. N. URBAN[1,4,5,3];
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Abstract: Early sensory experience can dramatically shape the adult anatomy and function of sensory circuits. In the olfactory bulb (OB), odor exposure through odorized food (food/odor pairing) fundamentally changes the structure of the glomerular module in a receptor-specific manner. We previously showed that prenatal and early postnatal odor exposure using methyl salicylate not only increases glomerular volume as measured by the coalescence of OSN axons but also increases the number of mitral and tufted cells (MTCs) connected to activated glomeruli. Increasing MTC number of a given glomerulus may increase the total output of that glomerulus,

and increase the total strength of lateral inhibition mediated by connected cells. Odor experience may also alter strengths of synaptic connections throughout the OB circuit. Here, we analyze the functional impact of this long-term odor exposure paradigm on population activity of MTCs. We use viral vector expression of GCaMP6s in mitral cells (MCs) to measure changes in odor-evoked responses following prenatal and early postnatal odor exposure. Dams are fed with food scented with methyl salicylate, an odorant known to activate selective dorsal glomeruli in the OB. Previous work in rodents suggest that odor-evoked changes in MTC firing rates are smaller when the odor presented is familiar. However, in our preliminary results we observe that odor presentation elicits larger amplitude odor responses in MCs from animals that experienced food/odor pairing than from naïve control animals. Larger responses were observed to both low and high concentrations of the familiar odorant, methyl salicylate, but the increase was larger at higher concentrations of odor presented. Specifically, MCs in odor-exposed animals showed a steeper slope of the concentration response function that was specific to the paired odor (Kruskal-Wallis test with Dunn's multiple comparisons test, n=35 cells, mean±SD of ΔF/F, 1% vs. 10% methyl salicylate stimulus, 1.14±0.11 vs. 1.30±0.22, $p<0.05$), while control animals did not (n=13 cells, mean±SD of ΔF/F, 1% vs. 10% methyl salicylate stimulus, 1.07±0.01 vs. 1.09±0.05, n.s.). In contrast, MCs in both odor-exposed and control animals did not demonstrate different concentration-dependent increases in response amplitudes when presented with increasing concentrations of a novel odor, hexanal (odor-exposed: n=35 cells, mean±SD of ΔF/F, 1% vs. 10% hexanal stimulus, 1.42±0.30 vs. 1.61±0.43, n.s; control: n=13, 1.15±0.08 vs. 1.17±0.08, n.s.). These odor specific responses suggest that odor exposure through a food-based paradigm significantly changes sensitivity of mitral cell response in an unexpected way.

Disclosures: **A. Liu:** None. **N.N. Urban:** None.

Poster

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Program#/Poster#: 619.04/QQ4

Topic: D.04. Olfaction and Taste

Support: Medical Research Council UK (MC_UP_1202/5)

Francis Crick Institute

Title: Task dependent modulation of olfactory representation

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Abstract: A ubiquitous feature of sensory processing in the brain is the presence of feedback and neuromodulatory connections: in many areas such connections are as dense as the feed-forward components. As this likely endows the brain with powerful functions such as emphasizing the behaviourally relevant aspect of environmental stimuli, it is crucial to understand how they contribute to the local circuit operations, as well as its underlying circuit mechanisms.
So far a variety of modulatory effects have been described across animal models. In contrast to modulations induced by general physiological changes such as during running, modulations that occur when animals attend to specific stimulus attributes are likely to be distinct, involving different circuit mechanisms.
To investigate this, we used the mouse olfactory system, as it allows for rapid behavioural task changes, and because the primary sensory region, the olfactory bulb (OB), is easily accessible for physiological experiments and already a major recipient of feedback and neuromodulatory inputs.
We sought to study the odour representation in the OB when animals are engaged in different behavioural tasks. GCaMP6f was expressed via AAV1 in OB neurons and the signals imaged repeatedly through chronically implanted window, in particular from the glomerular layer in awake, head-fixed mice. The same set of neurons could be imaged over weeks, where day-to-day responses to odours in individual neurons remained stable.
Diverse changes in odour representations were observed in the OB neurons as the task nature changed, with 32.7 ± 7 % of neurons in a given field of view showing significant changes. This change was present on a behaviourally relevant timescale, with changes present at least in the first 600 ms of odour response. Furthermore, the change was odour-specific, and occurred in a way that emphasized the aspect of olfactory stimuli relevant to the task. This was true for both GABAergic (Vgat-expressing) and non-GABAergic neurons. Consistent with its role in emphasizing the task-relevant stimulus attributes, the fluctuations of neuronal representation correlated with behavioural performance.
Overall the results suggest that the nature of sensory representation can change with specific behavioural demands even in the very first stages of processing. This experimental approach provides a promising way with which to investigate mechanisms underlying stimulus specific modulation.

Disclosures: I. Fukunaga: None. **A.T. Schaefer:** None.

Poster

619. Olfactory Circuits and Behavior

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Topic: D.04. Olfaction and Taste

Support: This work was supported by grant DC003906 from the NIDCD to D.A.W

Title: Experience-dependent lateralization in rat olfactory system

Authors: *Y. COHEN[1,2], D. PUTRINO[3,4], D. A. WILSON[1,2];
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Abstract: Lateralization, or cortical asymmetry, is a multifaceted phenomenon occurring at both the structural and functional levels, and can be expressed both as a stable trait and/or dynamically during behavior and development. For example, the piriform cortex (PCX) of rats performing a two-alternative forced choice odor discrimination task shows experience- and state-dependent asymmetry in odor-evoked local field potential (LFP) oscillations and inter-hemispheric coherence (Cohen et al., 2015). Specifically, odor-evoked LFP beta oscillations in the left PCX are more robust during initial learning than the right PCX. In contrast, the right PCX shows beta band enhancement at the late stages of learning while the left PCX changes return to baseline. With over-training, this asymmetry in cortical activity disappears. Importantly, the design of this study (Cohen et al., 2015) was based on behavioral performance which allowed us to detect the physiological changes at different performance levels.
To further explore asymmetry in the olfactory system during learning, here we used four different approaches: 1) Direct manipulation of the olfactory inter-hemispheric pathway. The anterior commissure (AC) is the inter-hemispheric pathway between the bilateral primary olfactory cortices, amygdala and entorhinal cortex. We cut the fibers of the AC to examine if and how olfactory tasks are modified by impairing olfactory cross talk between hemispheres. 2) Using the same design as before (Cohen et al., 2015), we examined a possible lateralization in orbitofrontal cortex during the odor learning. 3) Utilizing unilateral optogenetic stimulation of cortical networks as conditioned stimuli in a fear conditioning paradigm, directly testing whether one hemisphere is superior to the other for this form of odor learning or recall. 4) Screening PCX for lateralization in molecular markers related to plasticity to begin to understand the mechanisms of this widespread cortical asymmetry related to odor learning. Our results suggest that inter-hemispheric communication is essential for normal odor learning, especially in a reversal task. The OFC, similar to the PCX shows strong performance-dependent asymmetry, though this OFC asymmetry has distinct features different from that in the PCX. Finally,

PKC_{gamma} expression, a marker for synaptic plasticity, shows asymmetry in PCX layer I, that matches the emergence and recovery of LFP asymmetry over the course of learning.

Disclosures: Y. Cohen: None. **D. Putrino:** None. **D.A. Wilson:** None.

Poster

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Topic: D.04. Olfaction and Taste

Support: Ricerca Locale UNITO 2014-2015

Title: Reorganization of accessory olfactory bulb circuits driven by mating signals at puberty

Authors: *S. TROVA[1,2], L. OBOTI[3], R. SCHELLINO[2], A. ZHANG[4], N. HARRIS[5], O. ABIONA[4], W. LIN[4], P. PERETTO[1];
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Abstract: As a way to facilitate mating and breeding cycles, female sexual maturation and receptivity are often facilitated by the presence of male odors in the environment. The vomeronasal system, the accessory olfactory bulb in particular, is one of the primary brain circuits translating these signals into adaptive behavioral or neuroendocrine responses. We previously reported that the amount of GABAergic granule interneurons integrating in the AOB of female mice increases of about 50% in response to male odor stimulations occurring after puberty. One possible explanation for the timely occurrence of this circuit rearrangement could be the need for this pathway to improve its sensitivity to new sensory stimuli once they become salient signals for mating and reproduction. This indeed might imply the addition of newborn interneurons in olfactory sub-circuits elaborating these signals. To explore this possibility, here we provide evidence of a tight correlation between the activity-dependent modulation of AOB neurogenesis and the onset of female cycling at puberty. We further show that only male stimuli produced by putative mating partners - unrelated and unfamiliar mature males - are affecting the survival of newborn neurons in the female AOB. Genetic identity of the donors or their early postnatal experience do not prevent this to occur, as shown by exposing mature female mice to cues from either kin-related or unrelated littermates. Moreover, peri-pubertal exposure of female mice to male odors results in increased interest (investigation time) for the familiarized stimuli.

Unexpectedly, this response is accompanied by a drastic decrease in sexual receptivity shown to their male donors. Concurrently, male odor familiarization results in higher responsiveness of newly generated AOB granule cells, as assessed through c-Fos expression analysis, thus confirming the active recruitment of AOB newborn cells into circuits responsive to the familiarized signals. Collectively these data show that the activity dependent rearrangement of GABAergic circuits in the AOB occurs as female mice being to display attractive responses to male cues after puberty: it is contextual to the onset of mating behaviors, it depends on the sexual maturity of the male donors and it concurs with the recruitment of adult born neurons into AOB circuits encoding mate preference. Interestingly, the addition of new GABAergic neurons into these circuits correlates with a change in the behavioral effects of the sensory stimuli they elaborate. The time-scale of this process is compatible with a direct involvement of AOB neurogenesis in shaping the vomeronasal responses to mating signals after puberty.

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Poster

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Topic: D.04. Olfaction and Taste

Support: NIH Grant DC015186

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Title: Weighting of antagonistic sensory pathways underlies plasticity in behavioral response valence to olfactory cues

Authors: ***A. H. HARTMANN**[1], N. D. DWYER[2], C. I. BARGMANN[3], P. SENGUPTA[1]; [1]Brandeis Univ., Waltham, MA; [2]Univ. of Virginia, Sch. of Med., Charlottesville, VA; [3]Howard Hughes Med. Inst., The Rockefeller Univ., New York, NY

Abstract: Animals must integrate sensory inputs with contextual information, such as internal state or external cues, to execute adaptive behavioral responses. An important goal of neuroscience is to determine how this information is encoded within the nervous system. Behavioral plasticity can stem from genetic, molecular, and circuit level changes in neural function, allowing the organism to thrive in a dynamic environment. The nematode *C. elegans* is

an advantageous model organism for interrogating the neuronal circuits and molecular pathways involved in sensory processing and integration. *C. elegans* responds precisely, but flexibly, to sensory stimuli of multiple modalities, and sensory responses have been shown to be modulated by the animal's experience and state. The ability to rigorously control environmental conditions and experience, together with the array of available experimental tools, makes *C. elegans* an excellent system in which to interrogate the mechanisms underlying context-dependent behavioral plasticity.
I have found that antagonistic sensory pathways converge to mediate the behavioral response of *C. elegans* to the volatile odorant, hexanol. Specifically, the AWC sensory neurons are required for worms to exhibit attraction to hexanol, while the ASH/ADL nociceptive neurons appear to facilitate behavioral aversion to this chemical. Consistently, both AWC and ASH show calcium responses to removal and addition of hexanol, respectively. Differential weighting of these antagonistic pathways as a function of context and experience appears to drive flexibility in the animal's behavioral response to hexanol. I have found that environmental conditions such as population density alters the balance between these pathways; these behaviors can be recapitulated by genetic manipulations that specifically affect either the attractive or aversive circuit. I aim to identify the genes, neurons and circuits that alter response valence to olfactory cues, thereby describing how behavioral plasticity is encoded within a neural ensemble.

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Poster

619. Olfactory Circuits and Behavior

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Topic: D.04. Olfaction and Taste

Support: JSPS Grant-in-Aid for JSPS Fellows 14J06037

Title: Compartmentalized modulation of sensory and interneuronal activities for odor adaptation in *C. elegans*

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Abstract: Neuronal compartments are characterized by distinct physical-chemical characteristics and functional roles. The modulations of dendritic compartments are recently reported in some animals (Augustine *et al.*, 2003; Losonczy *et al.*, 2008; Cichon and Gan, 2015; Ogawa and Oka,

2015; Prešern *et al.*, 2015), but modulations of these dendritic compartment modulations for odor adaptation remains unclear in *C. elegans*. We, therefore, identified that compartmentalized modulations on sensory neurons and interneurons for odor adaptation with fluorescent imaging techniques. A 5-min odor pre-exposure reduces the odor attraction of worms. This phenomenon, which is named early adaptation, requires AWC sensory neurons and AIY interneurons (Hirotsu and Iino, 2005; Yamada *et al.*, 2009). However, modulations between AWC and AIY neurons have not been examined yet. Because AWC senses odors, and AIY receives inhibitory glutamatergic inputs from AWC, we investigated the modulations of neuronal activities with genetically encoded Ca^{2+} and glutamate indicators *in vivo*.Simultaneous Ca^{2+} imaging of AWC and AIY revealed that neuronal activities did not change in AWC neurons on pre-exposure. On the other hand, AIY neurons showed region-specific modulations of neuronal activity. Glutamate inputs to AIY also showed compartmentalized modulations. This study is the first reports of compartmentalized modulations of glutamate (input) and neuronal activity (output) on interneurons in *C. elegans.* Our results propose new insight for neuronal circuit study in *C. elegans.*

Disclosures: **K. Ashida:** None. **H. Shidara:** None. **K. Hotta:** None. **K. Oka:** None.

Poster

619. Olfactory Circuits and Behavior

Location: Halls B-H

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Program#/Poster#: 619.09/QQ9

Topic: D.04. Olfaction and Taste

Title: Perceiving and responding to stochastic olfactory stimulus in the *Drosophila* larva

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Abstract: The *Drosophila* larva navigates odor gradients by alternating runs with turns: they run up the gradient and turn while moving down the gradient. So far, deterministic stimuli have been used to study the control of larval chemotaxis. Little is known about the behavioral responses elicited by stochastic olfactory stimuli analogous to those encountered in nature. We address this problem by studying the control of reorientation decisions under sensory uncertainty (noise) during larval chemotaxis. We express the light-gated ion channel Channelrhodopsin-2 in olfactory sensory neurons (OSNs). Activating the OSNs with light allows us to stimulate larvae with a precision and reproducibility that is impossible to achieve with real odors. We mimic up- and down-gradient olfactory experiences and reproduce run-to-turn transitions by introducing

temporal ramps of increasing and decreasing light intensities. By quantifying the timing of run-to-turn transitions as a reaction time, we find that larvae exhibit robustness against weak Gaussian white noise corrupting deterministic olfactory stimuli. In the presence of strong noise, larvae show a delay (<1 sec) in their mean reaction time compared to the noise-free condition. However, strong noise does not impair chemotaxis severely. Finally, we describe the underlying decision-making process by applying a model commonly used in cognitive neuroscience: a diffusion model coupled with a linear-nonlinear model fed with OSN activity predicted from the stimulus. Our model suggests that invertebrates are capable of accumulating sensory evidence to enhance the robustness of perceptual decisions driven by noisy sensory evidence.

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Poster

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Topic: D.04. Olfaction and Taste

Support: Medical Research Council (MC_UP_1202/5)

Boehringer Ingelheim Fonds

Francis Crick Institute

Title: Learning-related changes in mitral and tufted cell responses reflect changes in sniffing behaviour

Authors: ***R. JORDAN**[1,2], I. FUKUNAGA[1], M. KOLLO[1], A. T. SCHAEFER[1,2];
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[2]Dept. of Neuroscience, Physiol. and Pharmacol., Univ. Col. London, London, United Kingdom

Abstract: Sensory circuit activity has often revealed correlates of information about behavioural context. These representations are generally thought to be driven by top-down input from higher centres, however it is conceivable that they could be evoked largely by learned changes in active sampling behaviours, e.g. sniffing or whisking. The olfactory bulb (OB) is ideal for mechanistic study of contextual modulation, with abundant input from higher centres, strong modulation of neural activity by sniffing, and the propensity for rapid learning of olfactory tasks in mice. Thus, we investigated the origin of contextual changes of mitral and tufted cells (MTCs) using whole cell recordings in awake mice, either passively exposed to odours (n = 21) or engaged in olfactory go/no-go task learning (n = 20 cells). While general physiology of MTCs between the

two behavioural states was similar (R_{inp},τ_{mem}, firing rate, V_{rest}, and odour responses, $p > 0.05$, t-test), response changes in learning mice were twice as numerous and significantly more diverse than in controls (0.2 ± 1.5 mV, vs -0.3 ± 1.0 mV; $p < 0.05$, Bartlett test). The learning-specific changes largely comprised increases in excitatory response. Notably, sniffing behaviour also underwent a variety of task-related changes (mean inhalation duration -2 ± 25 ms, vs 1 ± 10 ms; $p < 10^{-4}$, Bartlett test) depending on the motivational state of the animal (quantified by the anticipatory lick rate, $R^2 = 0.5$, $p < 0.005$). Both sniff and response changes occurred prior to earliest estimates of decision time (170 ms), making them potentially relevant for behaviour. Indeed, learning-related changes in sniffing showed strong relationships with changes in odour response, particularly in responses undergoing increases in excitation (R^2 up to 0.7, $p < 10^{-7}$). Correlating sniff change and Vm change across all putative mitral cells showed that changes in sniff rate alone accounted for almost 40% of variance in odour responses ($R^2 = 0.38$, $p = 0.001$). Sniff behaviour could modulate membrane potential by up to $\Delta Vm = 2.5$ mV in absence of either odour or learning when sniff rate changes were e.g. evoked by unexpected non-olfactory stimuli. The magnitude of ΔVm strongly depended on theta modulation properties of the cell ($R^2 = 0.37$, $p = 3 \times 10^{-4}$) and its odour response ($R^2 = 0.63$, $p = 0.003$), supporting a causal link between sniff changes and changes in membrane potential and odour responses. Overall, we conclude that contextual modulation of sensory circuit activity can be driven, at least in part, simply by active changes in stimulus sampling behaviour rather than necessarily being a consequence of top-down inputs or plasticity in the olfactory bulb.

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Poster

619. Olfactory Circuits and Behavior

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Topic: D.04. Olfaction and Taste

Support: CL was funded as a visiting professor to the University of Lyon 1

Title: Stabilization of olfactory learning by noradrenergic modulation in the olfactory bulb

Authors: ***C. LINSTER**[1], Y. THENNAISIE[2], M. MIDROIT[2], X. YIN[2], J. FOREST[2], M. RICHARD[2], A. DIDIER[2], N. MANDAIRON[2];
[1]CPL & Neurobio. and Behavior, Cornell Univ., Ithaca, NY; [2]Neuroplasticity and Neuropathology of Olfactory Perception, INSERM/CNRS, Lyon Neuroscience Research Center, LYON, France

Abstract: Among other central inputs, the olfactory bulb receives abundant noradrenergic (NE) projections from the locus coerulus (LC). These modulatory inputs have been shown to regulate olfactory bulb processing through changes in excitability, dynamics and plasticity. Specifically, NE in the olfactory bulb has been associated with low concentration odor detection, signal to noise modulation and discrimination of highly similar odorants. Previous studies used pharmacological or lesioning methods to investigate the role of NE modulation in the olfactory bulb, which puts temporal constraints on the behavioral paradigms that are possible. We use optogenetic inactivation of NE inputs to the olfactory bulb to manipulate NE modulation on a shorter temporal times scale. Briefly, mice were injected with Lenti-hSyn-NpHR-EYFP in the LC and simultaneously implanted with bilateral optical fibers in the olfactory bulb. After time for expression of the NpHR-EYFP in LC terminals in the OB, mice were trained to perform a simultaneous go-no-go task for 20 trials massed in a day, and then tested for recall of the odor discrimination either 4 hours or 24 hours after the training session during 5 trials. We found that decreasing the release of NE in the olfactory bulbs during learning of an odor discrimination has no impact on learning and recall after 4 hours, but severely impaired recall after 24 hours. These findings suggest a role of NE release in the OB not for expression of learning but for the maintenance of the long term memory. To further test this hypothesis, we tested these mice on a reversal learning. Mice were trained on a given odor contingency for 10 trials (with or without blockade of NE inputs), after which the contingency of odors was switched. Mice with impaired NE inputs learned the reversal significantly faster than controls, further suggesting that the formed memory is more labile when NE is decreased in the OB. Further experiments showed that the impact of NE on memory stabilization would be mostly due to activation of alpha1 receptors. All together, these data highlight a role of bulbar NE projections for the expression of olfactory memories. Long term maintenance of these memories is affected even when NE release is manipulated only temporarily during learning.

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Poster

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Topic: D.04. Olfaction and Taste

Support: PAIFC, Facultad de Ciencias

Fondecyt 1151432

Title: Ontogenic development of morphometric heterogeneities between vomeronasal pathways: the social rodent Octodon degus as a case in point.

Authors: P. FERNANDEZ-ABURTO, S. DELGADO, K. BULDRINI, G. MARIN, *J. MPODOZIS;
Univ. of Chile, Santiago, Chile

Abstract: In mammals, the vomeronasal system (VNS) has been largely associated with the coordination of social and sexual interactions. This system detected mainly pheromone-like semiochemicals, orchesting the neuroendocrine and behavioral responses of the organisms in a context-dependent manner. The VNS features two types of chemosensitive neurons segregated in the vomeronasal organ (VNO), each expressing either V1R or V2R receptor. In turn, vomeronasal neurons extend its axons to glomeruli located in the accessory olfactory bulb (AOB), forming two segregated subdomains: anterior (aAOB) and posterior (pAOB), respectively. Secondary projection neurons of both subdomains innervate multiple forebrain regions mainly associated to neuroendocrine regulation. Functionally, it has been suggested that V1R neurons detect small and volatile molecules while V2R neurons detect high and non-volatile molecules. It has been recently shown that several species of caviomorph rodents exhibit marked differences in morphometric parameters between AOB subdomains. Both, aAOB or pAOB-bias has been observed in these species, in association with conspicuous differences in life-style traits (semiarid vs semiaquatic environments). Whether and to which extent these differences are innate or arise in an experience dependent manner remains unexplored. We studied the ontogeny of the VNS in the hystricognath rodent *Octodon degus.* At adult stage, *O. degus* exhibit a highly heterogeneous AOB, in which the aAOB have twice overall volume than the pAOB, and features more and larger glomeruli. We found that segregated projections of the VNO neurons to its corresponding AOB subdomains were clearly distinguishable at prenatal stages. However, at the AOB the anatomical organization and the expression of neural maturation markers reached an adult pattern only from P15. Measurements of the overall AOB volume start showing a bias towards the aAOB by P15, but this bias becomes evident at the glomerular layer (GL) only by P30. This bias increase in posterior stages, reaching an adult-like pattern only at P180. In addition, and most interestingly, we found that these morphometric differences were less marked in GL for animals raised in captivity when compared with animals raised in the wild. Furthermore, we found that these morphometric differences were absent in the GL of *O. lunatus,* a sister-group that exhibit a low extent of social behavior. We conclude that morphometric differences between vomeronasal pathways arise in postnatal stages, suggesting that active semiochemical experience associated with the social context may be influencing the establishment of this heterogeneity.

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Poster

619. Olfactory Circuits and Behavior

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Topic: D.04. Olfaction and Taste

Title: Quantitative genetic differences in the olfactory bulbs of forebrain-specific Ctgf knockout mice

Authors: *H.-C. CHANG[1], L.-J. LEE[1,2,3];
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Abstract: Connective tissue growth factor (CTGF) is indispensable in the development of the connective tissue. In the nervous system, CTGF is expressed in some distinct areas, such as the olfactory bulb, but its function is largely unknown. In order to investigate the role of CTGF in the nervous system, forebrain-specific Ctgf knockout (FbCtgf KO) mice were made. In FbCtgf KO mice, altered neuronal patterning in the glomerular layer of olfactory bulbs and abnormal olfaction-related behavior were noticed. To pinpoint the molecular mechanism underlying these behavioral and cytoarchitectural changes, we analyzed the gene expression profiles in the olfactory bulb using next-generation RNA sequencing techniques. Unexpectedly, the expression of genes involved in the TGF-beta pathway which is downstream of CTGF signaling were not significantly changed. Besides related genes of TGF-beta signaling pathway, about 29 genes and their isoforms were significantly downregulated. Most of them take part in cell proliferation, cell migration, cell differentiation and cell death. Interestingly, the expression levels of Erythroid differentiation regulator 1 (Erdr1), myosin heavy chain 14 (Myh14) and Gm16286, were increased significantly. Erdr1 regulates cell proliferation, cell migration, and the maintenance of cell population. Myh14 is required in controlling of cytoskeleton activities and the maintenance of cell shape. Both of Erdr1 and Myh14 are highly expressed in olfaction-related brain regions. According to relative expression pattern and biological function, these genes may closely interact with CTGF. Our data widen the view to explore the function of CTGF in the nervous system.

Disclosures: H. Chang: None. **L. Lee:** None.

Poster

619. Olfactory Circuits and Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 619.14/QQ14

Topic: D.04. Olfaction and Taste

Support: NIH Grant DC-00338

Title: Developmental myelination of the lateral olfactory tract and anterior commissure

Authors: L. N. COLLINS[1], *P. C. BRUNJES[2];
[1]Psychology, Univ. of Virginia, Charlottesville, VA; [2]Dept Psychol, Univ. of Virginia Dept. of Psychology, Charlottesvle, VA

Abstract: The olfactory system employs two large myelinated tracts: the lateral olfactory tract (LOT), comprised of mitral/tufted cell axons coursing from the olfactory bulb to the ipsilateral olfactory cortices, and the anterior commissure (AC) which coordinates olfactory information between the left and right hemispheres. The current work is a comparison of the development of myelin in the two tracts of the mouse. Oligodendrocyte maturation was visualized by quantifying cells expressing markers for a) the oligodendrocyte lineage: Olig2, b) proliferative precursor cells: PDGF, c) intermediate pro-oligodendrocytes: CC1 and NG2 and d), mature, myelinating oligodendrocytes: MBP and MOBP at postnatal days (P)10, 20, and 30. In the LOT the late markers MBP and MOBP were already heavily expressed by P10. In the AC, early markers (PDGF, NG2, CC1) were prevalent at P10. By P20 their expression was decreased and both MBP and MOBP were abundant. Electron microscopy was used to track the proportion of myelinated to unmyelinated axons as well as axon caliber. Myelinated axons did not appear in the AC until P15; by P30 18% of axons were myelinated. These results suggest that myelination begins earlier in the LOT than the AC. More focused work confirmed that the LOT is myelinated 3-4 days prior to the AC and that both the AC and LOT exhibit a period of rapid myelination (LOT: P8-11; AC: P11-15). Additionally, the effects of sensory deprivation on the myelination of the AC and LOT were examined. Pups underwent unilateral naris occlusion on P1 and were reared until P30. EM analysis revealed that naris occlusion did not significantly change the number of myelinated axons in the AC relative to that of controls, but did reduce the axon caliber of myelinated fibers. The results suggest that the LOT and AC have different developmental histories and that sensory deprivation affects axon caliber of fibers carrying olfactory information

Disclosures: L.N. Collins: None. **P.C. Brunjes:** None.

Poster

619. Olfactory Circuits and Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 619.15/QQ15

Topic: D.04. Olfaction and Taste

Title: Apolipoprotein E4 mediates olfactory network excitability and short term habituation

Authors: *K. PENG[1,3,4], P. M. MATHEWS[1,5], E. LEVY[1,5,4], D. A. WILSON[2,3,6];
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Abstract: While expression of the apolipoprotein E ε4 allele (ApoE4) is well appreciated as the greatest genetic susceptibility factor for Alzheimer's disease (AD), more recent findings suggest that ApoE4 expression leads to cognitive deficits, neurodegenerative risk and brain atrophy independently of AD. In human ApoE4 carriers, the onset of impaired odor identification correlates with the onset of episodic memory decline. Olfactory perception relies upon the spatiotemporal pattern of transfer of odor-evoked activity in the olfactory bulb (OB) to the piriform cortex (PCX). From the PCX, odor information travels to the entorhinal cortex and hippocampus for future retrieval.
We examined olfactory behavior and physiology in 6 and 12 month old mice humanized for ApoE4 compared to mice humanized for the common neutral-risk ε3 allele (ApoE3), neither of which develops AD-related amyloid pathology. Testing odor habituation, a measure of short-term memory, we found deficits in ApoE4 compared to ApoE3 mice at 6 months of age, although this behavioral deficit did not persist into older age groups. Given that the synaptic mechanisms of short-term habituation originate within the connections between the OB and the PCX, we recorded spontaneous and odor-evoked local field potentials (LFPs) in both regions from anesthetized mice. At 6 months of age, no genotype differences in spontaneous LFPs without the presence of odors were seen in either region. However, odor presentation triggered hyperactive LFPs in the OB of 6 month old ApoE4 compared to ApoE3 mice. At this age, no odor-evoked genotype-dependent differences in LFPs were found when recording from the PCX. At 12 months of age, spontaneous LFPs were found to be hypoactive in the PCX, but not the OB, of ApoE4 mice compared to ApoE3 mice. Meanwhile, the presence of odor at 12 months triggered an age-dependent increase in evoked LFPs activity in the PCX of ApoE4 mice.
Thus, regional olfactory system function is impaired in an age- and ApoE genotype-dependent manner. Our findings suggest that early olfactory network abnormalities in ApoE4 OB coinciding with short-term memory impairments may be a precursor to later network dysfunction in the PCX. Further studies are required to determine the extent to which evoked

hyperexcitability spreads throughout the brain and whether changes in circuit activity may compromise other cognitive tasks in ApoE4 carriers.

Disclosures: **K. Peng:** None. **P.M. Mathews:** None. **E. Levy:** None. **D.A. Wilson:** None.

Poster

619. Olfactory Circuits and Behavior

Location: Halls B-H

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Program#/Poster#: 619.16/QQ16

Topic: A.10. Development and Evolution

Support: MOST, 104-2311-B-001-031-mY3

Title: Regulation of interneuron distribution by the fate of cortical projection neurons

Authors: ***Z.-X. NIOU**, H.-W. HSING, S.-J. CHOU;
ICOB, Academia Sinica, Nankang District, Taipei City, Taiwan

Abstract: The two major neuronal types in the mammalian cortex are the excitatory projection neurons and the inhibitory interneurons (INs). The projection neurons are generated in the dorsal telencephalon (dTel) and migrate to their destination by radial migration. The GABAergic interneurons are primarily generated in the medial ganglionic eminence (MGE) of ventral telencephalon (vTel) and enter the cortex by tangential migration. Here, using a panel of IN markers, we demonstrate that although the IN density is consistent in different regions in the cortex, the IN distribution patterns are different in the six-layered neocortex and in the three-layered piriform cortex. To study how IN distribution is regulated, we used Lhx2 conditional knockout (cKO) mice, in which Lhx2 is deleted by Emx1-Cre, as a model. In Lhx2 cKO cortices, the lateral neocortex is refated to generate an ectopic piriform cortex. We found in the ectopic piriform cortex, the density of INs is similar to that in the wild type cortex and the distribution of INs is similar to that in the PC in the wild type littermates. This findings suggest that the fate of projection neurons regulates the distribution of the INs.

Disclosures: **Z. Niou:** None. **H. Hsing:** None. **S. Chou:** None.

Poster

619. Olfactory Circuits and Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 619.17/QQ17

Topic: D.04. Olfaction and Taste

Title: Studies on olfactory neural circuit development mediated by local caspase activity and cIAP-1 during the mouse postnatal development.

Authors: *S. MOON, S. KIM, B. CHO, C. MOON;
DGIST, Daegu, Korea, Republic of

Abstract: Correct connections between neurons are directly linked to the precise function of our brain. Between the olfactory epithelium (OE) and the olfactory bulb (OB), there have simple neural connections which form the olfactory neural map. Incorrect coalescence of olfactory sensory neurons (OSNs) at the glomerulus could cause the problem such as the failure of odor encoding and discrimination. Therefore, making proper synapse and maintenance of olfactory neural connectivity is important during the postnatal development. Despite the significance of this process, only few studies about periods of axon path-finding, synapse formation and refinement have been reported in the olfactory system. Especially, the mechanism about the maintenance and remodeling of olfactory neuronal circuits is still unknown.
In this study, we hypothesized that there are critical periods and regulator(s) to establish the stable adult olfactory neural connections. To investigate this topic, we focused on caspases and the cellular inhibitor of apoptosis-1 (cIAP-1). Because recent studies indicated non-apoptotic roles of caspases such as neuronal plasticity, neuronal growth and regeneration, and degeneration of axons and dendrites. And cIAP-1 is one of the best known regulator of caspase function. To check whether cIAP-1 level is significant to modulate the olfactory neural connectivity or not, we checked the cIAP-1 expression level in the olfactory system by comparing WT mice to cIAP-1 KO mice. According to our preliminary results, cIAP-1 level was up-regulated until 7 weeks during the postnatal development stages. At this point, caspase 3 was activated when cIAP-1 was ablated. Using immunohistochemistry, we confirmed local caspases activity at the axonal part of OSNs in the cIAP-1 KO mice. These results suggest that cIAP-1 may inhibit the local activation of caspases, and which may stabilize the olfactory neural circuit during the specific periods of postnatal development.
Our studies would be helpful to understand how regenerated OSNs maintain the proper olfactory neural connectivity during the post-natal development.

Disclosures: **S. Moon:** None. **S. Kim:** None. **B. Cho:** None. **C. Moon:** None.

Poster

619. Olfactory Circuits and Behavior

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Topic: D.04. Olfaction and Taste

Support: NIH/NIMH 1RO1MH091348-01

Title: Role of extracellular matrix chondroitin sulfate proteoglycan expression in the human olfactory system connectivity

Authors: *F. J. HAMATI[1], K. K. PILOBELLO[1], A. BOYER-BOITEAU[1], H. PANTAZOPOULOS[1,3], C. BERCIU[2], E. H. HOLBROOK[5], S. BERRETTA[1,3,4];
[1]Translational Neurosci. Lab., [2]McLean Hosp., Belmont, MA; [3]Dept. of Psychiatry, [4]Program of Neurosci., Harvard Med. Sch., Boston, MA; [5]Dept. of Otolaryngy, Massachusetts Eye and Ear Infirmary, Boston, MA

Abstract: Chondroitin sulfate proteoglycans (CSPGs), one of the main components of the brain extracellular matrix, play an important role in axon guidance and modulation of synaptic stability during development. The olfactory system represents an ideal model to study these functions, as CSPGs are robustly expressed in this system and neuronal differentiation, synaptic stabilization, and axon outgrowth continue throughout life. Axons from olfactory receptor neurons (ORNs) travel across the olfactory mucosa (OM) and to odor-specific glomeruli in the olfactory bulb (OB). CSPGs may play guidance and stabilization roles which may be central to the discriminatory function of the GLs in the OB. Thus, we postulated that CSPGs form organized extracellular structures surrounding ORN axons, potentially guiding them from the OE the correct odor-specific OB glomeruli.
Human post-mortem OBs (n=10, age- and sex-matched), rodent OM (n=8) and OM tissue biopsies from healthy human subjects (n=15) were used for immunocytochemistry and electron microscopy studies. An antibody (CS56) raised CSPGs with a CS-6 sulfation pattern and the lectin *Wisteria Floribunda agglutinin* (WFA) were used to investigate the relationship between distinct CSPGs with ORN axons labelled using anti-olfactory marker protein antibody.
Our results show that, in human, CSPG/CS-6 are closely associated with ORN axons, forming dense aggregates subjacent the olfactory epithelium (OE), and deeper channel-like structures which encapsulate ORN axon bundles, following them from the OE, through the OM and into the glomerular layer of the OB. Other CSPGs, labeled with WFA, are associated with the medial side of the glomeruli and throughout the OB's inner layers. Consistent with these results, electron micrographs of rodent OM show extracellular CSPG/CS-6 aggregates deposited around ORN axon bundles.
These findings are consistent with the hypothesis that CSPGs play a key role in organizing and

guiding ORN axons from the OE into OB glomeruli and maintaining the glomerular structure and connectivity with the tufted and mitral cells. These results are relevant to the pathophysiology of schizophrenia (SZ), a disorder with CSPG expression abnormalities in several brain regions including the OE. Notably, people with SZ, and first-degree relatives, present with odor identification deficits. We suggest that CSPG abnormalities in the olfactory system of individuals with SZ may disrupt the guidance of ORN axons, thus destabilizing connections within the odor-specific glomeruli contributing to the observed olfactory deficits.

Disclosures: **F.J. Hamati:** None. **K.K. Pilobello:** None. **A. Boyer-Boiteau:** None. **H. Pantazopoulos:** None. **C. Berciu:** None. **E.H. Holbrook:** None. **S. Berretta:** None.

Poster

620. Spatial Factors of Crossmodal Integration

Location: Halls B-H

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Topic: D.09. Multisensory Integration

Support: JSPS KAKENHI 26700012

JSPS KAKENHI 15K12615

JSPS KAKENHI 15K17333

MEXT KAKENHI 15H01590

Title: Salivary oxytocin concentration is correlated with the subjective feeling of body ownership during the rubber hand illusion

Authors: ***M. IDE**[1,2], M. WADA[1];
[1]Dev. Disord. Sect., Dept. of Rehabil. for Brain Functions, Res. Inst. of NRCD, Tokorozawa, Saitama, Japan; [2]Japan Society for the Promotion of Sci., Chiyoda, Tokyo, Japan

Abstract: Oxytocin is known to be a neuropeptide that promotes lactation, maternal bonding and birth. Recent studies have reported that oxytocin modulates social recognition and thus seems to be related to empathic behaviours. Multiple studies have suggested that the oxytocin modulates social brain networks, which are related to empathy (e.g., insular cortex). These networks are also activated during the rubber hand illusion (RHI), which is an illusory body ownership caused by the brush stroking that were synchronously applied to a rubber hand and a participant's hand. It is intriguing to investigate whether oxytocin affects illusory changes in the body ownership, such as in the RHI. In this study, we examined the relationship between salivary oxytocin

concentration and the subjective feeling of rubber hand ownership in each participant. In the behavioural task (N = 10), brushes were stroking synchronously or asynchronously to the participant's hand and a rubber hand. Each condition included 4 sessions, and each session lasted 3 minutes. The synchronous and asynchronous stroking conditions were conducted on separate days (within 4 days). Saliva (2 ml) was sampled before and after the behavioural tasks, and oxytocin concentration was measured with ELISA method. We found that participants who had higher concentrations of salivary oxytocin tended to feel stronger ownership of the rubber hand ($r = 0.79$, $p = 0.0067 < 0.01$). In contrast, the salivary oxytocin concentrations did not change after the occurrence of the RHI in the present condition. Additional analysis showed that the participants with higher autistic traits tended to feel a weaker body ownership of rubber hand in the RHI task ($r = -0.71$, $p = 0.018 < 0.05$). Our results suggest that a salivary oxytocin concentration can predict degree of subjective feeling of body ownership during the RHI, and it might modulate our sensation of body ownership. We speculate that oxytocin modulate the illusory body ownership by affecting brain activity in the insular cortex and anterior cingulate gyrus, because these regions are also known to be related to body image and self-consciousness.

Disclosures: **M. Ide:** None. **M. Wada:** None.

Poster

620. Spatial Factors of Crossmodal Integration

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

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Topic: D.09. Multisensory Integration

Support: KAKENHI

CREST,AMED

Title: Prediction error responses in the mouse posterior parietal cortex are dependent on molecular diversity of clustered protocadherin α

Authors: ***K. YOSHITAKE**[1,2], H. TSUKANO[1], R. HISHIDA[1], T. YAGI[3,2], K. SHIBUKI[1,2]; [1]Niigata Univ., Niigata, Japan; [2]CREST, AMED, Tokyo, Japan; [3]Osaka Univ., Osaka, Japan

Abstract: Predictive coding is a theoretical concept which helps us to understand higher brain functions. According to this idea, the brain predicts what is going to happen under a particular situation based on prior experience. Therefore, one of essential brain functions is to predict the coming sensory input, and to minimize the prediction error (difference between the prediction and actual sensory inputs). This prediction error minimization process must be dependent on past

experience and learning. Previously, we have reported that prediction errors between whisker and visual inputs were detected in the posterior parietal cortex (PPC) of mice. We recorded neuronal activities in PPC using flavoprotein fluorescence imaging. Visual stimulation with grating patters moving forward/backward alone, or forward/backward whisker-shift stimulation alone hardly activated PPC in anesthetized mice. However, anti-phase combination of moving grating patterns and whisker stimulation (grating moving forward plus backward whisker shift, or grating moving backward plus forward whisker shift), which is very unlikely in natural environment for mice, produced prediction error responses in PPC. In contrast, in-phase combination of grating patterns and whisker stimulation failed to produce any clear activity in PPC. We have reported that cortical depression and retinotopic map shifts were induced by prediction errors between visual and whisker inputs in the primary visual cortex (V1) of young mice that had worn a monocular prism goggle, suggesting that the prediction errors detected in PPC were used to reduce in the prediction errors at the V1 level. Obviously, prediction and prediction errors are expected to be dependent on past experience and learning. To test this possibility, we investigated the prediction error responses in PPC of dark-reared mice from birth to 4 weeks of age. As expected, the dark-reared mice could not show any prediction error responses in PPC. However, this dark reared effect was cancelled after the mice returned to a normal light-dark cycle for 2 additional weeks. These results suggest that prediction error responses in PPC require previous experience. Clustered protocadherins (cPcdhs) comprising cPcdh-α, β, and -γ, encode a large family of cadherin-like cell-adhesion molecules specific to brain. Both of the prism-induced depression in V1 and the prediction error responses in PPC to the anti-phase combination of visual and whisker stimulation were impaired in mice with reduced cPcdh-α diversity. These results strongly suggest that the molecular diversity of cPcdh-α is important for the experience-dependent PPC functions to produce prediction errors.

Disclosures: **K. Yoshitake:** None. **H. Tsukano:** None. **R. Hishida:** None. **T. Yagi:** None. **K. Shibuki:** None.

Poster

620. Spatial Factors of Crossmodal Integration

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Tab Williams Foundation

Title: Multisensory versus unisensory integration: cooperation vs. competition

Authors: *B. A. ROWLAND, J. W. VAUGHAN, D. ZHU, B. E. STEIN;
Neurobio. & Anat., Wake Forest Sch. of Med., Winston Salem, NC

Abstract: Although multisensory superior colliculus (SC) neurons are best known for their ability to integrate signals across modalities (multisensory integration), they also integrate cues within a given modality (unisensory integration) - but these yield different products. Whereas two spatiotemporally concordant cross-modal cues evoke an enhanced physiological response, two synchronized and spatially proximal within-modal (e.g., visual) cues typically yield no enhancement. The response is no greater than the response to one of the cues alone. It is as if the unisensory integrative mechanism "discards" one of the two inputs. This contrast in the multisensory and unisensory computations is poorly understood. One possibility is that the non-enhancement in unisensory "integration" merely reflects the limited bandwidth available within a single sensory channel. However, the present study supports the idea that integration is taking place in both cases but using entirely different computational mechanisms. Individual multisensory SC neurons were tested with multiple spatially-aligned visual (V1, V2) and auditory (A) cues presented individually and in combination at multiple stimulus onset asynchronies. Responses to the cues presented individually (V1, V2, A) and in different combinations (V1A, V2A, V1V2) were compared in a moment-by-moment analysis. Whereas multisensory integration characteristically evoked large enhancements early in the response window (the IRE, or initial response enhancement), responses to within-modal pairs (unisensory integration) were equivalent to those evoked by an individual cue at each moment in time. When the within-modal cues (i.e., V1V2) were separated in time, the response profiles revealed the engagement of a competitive dynamic that actively suppressed responses to the delayed cue in the pair, resulting in an overall response that was not enhanced. Interestingly, similar products are obtained with cross-modal cues when a neuron's multisensory integrative capability is impaired. These observations indicate that unisensory integration, like multisensory integration (see accompanying posters by Cuppini et al., and Yu et al.), has a native competitive computation that is engaged when there are multiple inputs. Only in the case of multisensory integration is this native state overridden by experience during development. The result is the transition from competition to cooperation in the maturation of multisensory integration.

Disclosures: B.A. Rowland: None. **J.W. Vaughan:** None. **D. Zhu:** None. **B.E. Stein:** None.

Poster

620. Spatial Factors of Crossmodal Integration

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Topic: D.09. Multisensory Integration

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Tab Williams Foundation

Title: Superadditivity: a defining characteristic of multisensory integration?

Authors: *B. E. STEIN, R. L. MILLER, B. A. ROWLAND;
Neurobio. and Anat., Wake Forest Sch. Med., Winston Salem, NC

Abstract: Multisensory neurons in different regions of the brain integrate concordant information from multiple sensory sources to enhance responses. This often amplifies the physiological salience of biologically significant events and the likelihood of adaptive responses. Model areas in which this has been studied in the cat include the midbrain (i.e., the superior colliculus, SC) and cortex (the anterior ectosylvian sulcus, AES). The responses to concordant cross-modal cues in both regions can exceed the sum of the responses to the individual component cues (superadditivity); however, prior work suggests that such products are associated with brief, weakly-effective cues. More recently it has been shown that the products of multisensory integration are not uniform throughout the course of a response, but are largest near its onset, a region termed the initial response enhancement (IRE, see Rowland et al., 2007). The IRE coincides with a period in which the behaviors mediated by these circuits (detection, orientation, localization) are typically effected. To determine whether, in fact, superadditivity in the responses of SC and AES neurons was strictly associated with weakly-effective brief cues, or was a more general characteristic of the multisensory transform, the present study examined SC and AES neuronal responses to a wide variety of cross-modal stimulus combinations. The results demonstrate that a superadditive IRE is a near-ubiquitous phenomenon in both SC and AES neurons. It was present regardless of whether or not the cross-modal component stimuli employed were highly efficacious, and regardless of the physical properties of these stimuli (e.g., moving/stationary, short/long duration, small/large, high/low intensity). These observations challenge the notion that superadditivity is only reliably linked to brief, weakly-effective cues and reveal that superadditivity (within the IRE) is a hallmark property of multisensory integration. The results suggest that the IRE may play a larger sensory and sensorimotor role than is currently appreciated.

Disclosures: **B.E. Stein:** None. **R.L. Miller:** None. **B.A. Rowland:** None.

Poster

620. Spatial Factors of Crossmodal Integration

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Topic: D.09. Multisensory Integration

Support: NIH Grant EY024458

Tab Williams Foundation

National Natural Science Foundation of China (Grant No. 31300910)

Title: Cross-modal competition: the native multisensory computation

Authors: ***L. YU**[1], J. XU[1], B. A. ROWLAND[2], B. E. STEIN[2];
[1]East China Normal Univ., Shanghai City, China; [2]Wake Forest Sch. of Med., Winston-Salem, NC

Abstract: Multisensory neurons in the superior colliculus (SC) typically integrate spatiotemporally concordant cross-modal cues in a synergistic fashion. However, this capacity for "multisensory enhancement" only develops after experience with covariant cross-modal cues. Prior work has suggested that, in the absence of this experience, the native multisensory computation is one that either reflects only the most efficacious component stimulus of a cross-modal pair or an average of the responses to the two unisensory inputs. The present study investigated this phenomenon in visual-auditory SC neurons of animals reared in complete darkness (precluding visual-nonvisual experience). The results show that the native multisensory computation is neither a "winner take all," nor an “averaging” operation, but a competition between the two inputs. This competitive mechanism was revealed only when two stimuli of very different effectiveness were combined, and the potency of their competition became more evident as the difference in their effectiveness increased. At very different effectiveness levels the multisensory response became significantly lower than the most effective unisensory comparator response, revealing an operation very much like the inhibitory (i.e., competitive) interactions between spatially disparate stimuli seen in normally-reared animals. In short, all multisensory SC neurons at all stages of life may be capable of integrating cross-modal cues, but with different operational principles. The multisensory maturational process, via the acquisition of cross-modal experience, appears not to reflect the construction of a computation from a “blank slate,” but rather a transition from one of competition to one of cooperation. In this way SC neurons, and presumably multisensory neurons elsewhere in the brain, become capable of identifying the common origin of cross-modal signals. By then enhancing their access to the sensorimotor circuitry of the SC, they also enhance the likelihood of an SC-mediated adaptive response.

Disclosures: **L. Yu:** None. **J. Xu:** None. **B.A. Rowland:** None. **B.E. Stein:** None.

Poster

620. Spatial Factors of Crossmodal Integration

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Topic: D.09. Multisensory Integration

Support: Italian Ministry of Education, Project FIRB 2013 RBFR136E24

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Title: The specificity in multisensory learning reveals a native competitive interaction among sensory representations.

Authors: ***C. CUPPINI**[1], M. URSINO[1], E. MAGOSSO[1], B. A. ROWLAND[2], B. E. STEIN[2]; [1]Univ. of Bologna, Bologna, Italy; [2]Dept. of Neurobio. and Anat., Wake Forest Univ. Sch. of Med., Winston-Salem, NC

Abstract: The midbrain superior colliculus (SC) integrates information from different senses (visual, auditory, somatosensory) in mediating attentive and orientation behaviors. Its trisensory neurons are able to integrate information across each of the possible pairings of these modalities, thereby facilitating this role to a variety of biologically relevant events. However, these integration capabilities are not an inherent characteristic of the SC neuron; rather, they are acquired during postnatal life based on experience with cross-modal cues. Recent evidence has revealed that neurons do not generalize their experience-based multisensory integration capabilities across modality-pairings. For example, experience with visual-somatosensory cues confers the ability to integrate information from these modalities, but not from visual-auditory cue pairs. Thus, neurons "learn" to integrate information only from those cross-modal pairs with which they had explicit experience (Xu et al., 2015). This learning also requires functional inputs from association cortex (i.e., the anterior ectosylvian sulcus, AES) (REF). Here we describe an artificial neural network model that explains how these phenomena can be implemented within the AES-SC circuit. The model is based on a few simple assumptions: 1) all modality-specific inputs to SC are mutually competitive by default, 2) tectopetal inputs arising from AES are trained by cross-modal experience to bypass this competition, consequently interacting in cooperative ways, 3) this training obeys simple Hebbian learning rules, and 4) after training, SC responsiveness becomes dominated by these AES inputs. The model was tested for accuracy by

repeatedly simulating exposure to auditory, visual, and somatosensory stimuli alone or combined in all possible modality-pairings. It accurately replicated the empirical findings and suggests that the native state is one of cross-modal competition.

Disclosures: C. Cuppini: None. **M. Ursino:** None. **E. Magosso:** None. **B.A. Rowland:** None. **B.E. Stein:** None.

Poster

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Topic: D.09. Multisensory Integration

Support: FP7 EU STREP 611621

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Title: Seeing with ears, how we create an auditory representation of space with echoes and its relation with vision.

Authors: *A. TONELLI[1], L. BRAYDA[2], M. GORI[1];
[1]U-VIP Unit for Visually Impaired People, [2]Robotics, Brain and Cognitive Sci., Fondazione Inst. Italiano Di Tecnologia, Genoa, Italy

Abstract: We will present an overview of our works that explore the relation between vision, auditory space perception and alternative ways to compensate for the lack of vision. Several studies show that vision is essential in the domain of space perception influencing also other sensory modalities, a proof is the Ventriloquist effect. Vision can interact with audition even when a visual stimulus is not provided during an auditory task, but just observing the setup with eyes open can improve audio accuracy. Interestingly we found that the improved performance in complex auditory space task occurs already after a brief observation of the environment performing the task blindfolded. These findings suggest that vision is important on an on-line evaluation of complex auditory cues thanks to an internal representation. Moreover, this representation appears to be useful in reverberant environments helping to compensate for the mismatching due to the reverberation itself. Another example for the importance of vision in space perception is provided by blind people whereas there were found impairments in performing complex spatial auditory tasks. These results suggest that the visual system plays an important role during development calibrating the audio system in space perception. However these impairments are not present in blind people whom have developed echolocation.

Echolocation is the ability to understand the environment through echoes thanks mainly self-generated clicks. It has also been shown that echolocation, other than help to have better spatial abilities, contributes to functional benefits in real life. Therefore echolocation seems to compensate for the lack of vision. For these reasons we decided to teach echolocation to naïve sighted people. We started with sighted people testing them in some tasks of echolocation, such as depth or detection task. Moreover, we investigate if other than help to understand the external space, echolocation could influence the space surrounding the body, as for example the peripersonal space (PPS). Our data suggest that sighted people can learn echolocation after a brief training. Thanks to this training sighted people, without vision, are able to understand the environment and what there is inside. Moreover, echolocation can modify how the PPS is perceived. All this information demonstrated how human are sensible to echoes and that spatial information can be acquired not using vision, also in people that normally would acquire the same information through it. If also sighted people can echolocate it could be developed a targeted training to teach echolocation to blind people.

Disclosures: **A. Tonelli:** None. **L. Brayda:** None. **M. Gori:** None.

Poster

620. Spatial Factors of Crossmodal Integration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 620.08/RR6

Topic: D.09. Multisensory Integration

Title: Recalibrating the body: visuotactile ventriloquism aftereffect

Authors: ***M. J. SAMAD**, L. SHAMS;
Psychology, UCLA, Los Angeles, CA

Abstract: Background: The visuotactile ventriloquism effect is a recently reported effect showing that somatotopic tactile representations (namely, representation of location along the surface of one's arm) can be biased by simultaneous presentation of a visual stimulus in a spatial localization task along the surface of the skin. Aim: Here we investigated whether the exposure to discrepancy between tactile and visual stimuli on the skin can induce lasting changes in the somatotopic representation of space. Methods: We conducted an experiment investigating this question by asking participants to perform a localization task that included unisensory and bisensory trials, before and after exposure to spatially discrepant visuotactile pairs. Subjects localized brief flashes of light and brief vibrations that were presented along the surface of their forearms, and were presented either individually (unisensory conditions) or were presented simultaneously at the same location or different locations. We then compared the localization of

tactile stimuli in unisensory tactile conditions before and after the exposure to discrepant bisensory stimuli. Moreover, using the Bayesian causal inference model of multisensory perception, we examined whether any change in localization performance was best accounted for by a change in sensory representations, or a priori biases, or a combination of the two. Results: After exposure, subjects did indeed exhibit a shift in their tactile localizations in the direction of the visual stimulus that was presented during the exposure phase. Moreover, this recalibration was best accounted for by a shift in the tactile representations of space as opposed to a change to the prior expectation of the location of the stimuli. Conclusions: These results demonstrate that the somatotopic spatial representations are capable of rapidly recalibrating after a very brief exposure to spatially discrepant visuotactile stimuli. They also suggest that this change may be implemented at the level of the encoding of these tactile stimuli.

Disclosures: M.J. Samad: None. **L. Shams:** None.

Poster

620. Spatial Factors of Crossmodal Integration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 620.09/RR7

Topic: D.09. Multisensory Integration

Support: Donders Institute internal TopTalent grant for PhD candidate

Title: Allocentric and egocentric contributions to the updating of visual object orientation

Authors: *N. NIEHOF, J. J. TRAMPER, C. F. DOELLER, W. P. MEDENDORP;
Donders Institute, Radboud Univ., Nijmegen, Netherlands

Abstract: We tested the role of allocentric and egocentric spatial reference frames in the coding and updating of object orientation during head motion. When the body is stationary, allocentric visual information affects the perception of object orientation. For example, subjects make systematic errors in judging the verticality of a line (an estimate of the gravity direction), in the presence of a tilted visual frame. However, it is not yet clear how and to which extent subjects remember the orientation of this line after intervening head and frame rotation. We examined three types of errors subjects could make in this updating task. If subjects anchor the line to the visual frame, updating errors will depend on the magnitude of the intervening frame rotation. If subjects code the line relative to the head, updating errors will relate to the intervening head rotation. If subjects store the line relative to gravity, errors would be linked to their subjective vertical, which was measured separately. Ten subjects were asked to remember the orientation of a line stimulus, briefly flashed within a tilted frame, while the head was oriented at a clockwise

roll tilt of 30°. Subjects then rotated their head to upright, and made a two-alternative forced choice on whether the orientation of a second line, always flashed in an upright frame, was clockwise or counterclockwise relative to the initial line orientation. Furthermore, each subject's subjective vertical was determined through a verticality judgment of a single line in a tilted frame, in both the upright and tilted head position. Results show that subjects make systematic errors in the updating task. Regression analyses indicate that these errors were related to the subjective vertical and the head orientation, but showed no significant relation to the visual frame orientation. These findings suggest that subjects represent orientations both allocentrically, relative to gravity, and egocentrically, relative to the head in space. Our results support the notion that the brain uses multiple reference frames in parallel to code and update visual object orientations.

Disclosures: N. Niehof: None. **J.J. Tramper:** None. **C.F. Doeller:** None. **W.P. Medendorp:** None.

Poster

620. Spatial Factors of Crossmodal Integration

Location: Halls B-H

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Program#/Poster#: 620.10/RR8

Topic: D.09. Multisensory Integration

Support: CONACyT Fronteras de la Ciencia 536

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VIEP-PIFI-FOMES-PROMEP-BUAP-Puebla

FOMES-PROFOCIE BUAP and VIEP-BUAP 174101

Catedra Marcos Moshinsky

VIEP-BUAP MEBI-EDH-16

Title: Improving visual responses in the superior colliculus by tactile noise

Authors: *N. HUIDOBRO[1], B. DE LA TORRE-VALDOVINOS[2], I. MENDEZ-BALBUENA[3], E. MANJARREZ[4];
[1]Inst. de Fisiologia, Benemerita Univ. au, Puebla, Mexico; [2]Ciencias Computacionales, Cucei, Univ. de Guadalajara, Guadalajara, Mexico; [3]Facultad de Psicologia, Benemerita Univ.

Autonoma De Puebla, Puebla, Puebla, Mexico; [4]Inst. de Fisiologia, Benemerita Univ. Autonoma De Puebla, Puebla, Mexico

Abstract: Recently we demonstrated that an optimal level of tactile noise applied in the human finger increases the amplitude of EEG visual evoked potentials (Mendez-Balbuena et al., J Neurophysiol.114:2132, 2015). This finding suggested that such increase was produced by the synaptic interaction between multisensory neurons located in the superior colliculus. The aim of the present study was to examine, in an animal preparation, whether the multisensory neurons in the superior colliculus can reproduce such phenomenon. We obtained multiunit recordings of neurons from the superior colliculus in three pentobarbital-anesthetized cats. The visual evoked responses were elicited by the application of periodic stimuli with a pair of white LEDs. Simultaneously, we added three levels of continuous tactile noise on the central pad of the hindlimbs by means of a Chubbuck mechanical stimulator-transducer. The waveclus spike sorting software was employed to classify the unitary activity of collicular neurons. For all the subjects, we found that the total number of neuronal spikes measured from the peristimulus histogram exhibited an inverted U-like form as a function of the tactile noise level (i.e., we found that there is an intermediate optimal level of tactile noise producing an increase in the firing frequency of these collicular multisensory neurons). In order to compare the total number of spikes between zero noise (ZN), optimal noise (ON) and high noise (HN), we performed the one-way repeated measures ANOVA test to examine the statistical significance of the change in the total number of spikes between the three conditions (ON, ZN and HN) in the unitary responses of 85 neurons. The results showed significant differences between the three conditions ($p<0.0001$). The post hoc Tukey test revealed statistically significant differences between ZN and ON ($p<0.0001$) and between ON and HN ($p<0.0001$). In contrast, no significant differences were found between conditions ZN and HN. Based on these results it is tempting to speculate that the multisensory neurons located in the superior colliculus could contribute to the cross-modal stochastic resonance found in the human EEG recordings during the visuo-tactile interaction.

Disclosures: N. Huidobro: None. **B. de la Torre-Valdovinos:** None. **I. Mendez-balbuena:** None. **E. Manjarrez:** None.

Poster

620. Spatial Factors of Crossmodal Integration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 620.11/RR9

Topic: D.09. Multisensory Integration

Support: Human Frontiers Science Foundation RGY0068

Title: Frontal and auditory cortical activity in ferrets using selective attention in a multisensory task-switching paradigm

Authors: *S. M. TOWN, J. K. A. NORDMARK, J. K. BIZLEY;
UCL Ear Inst., London, United Kingdom

Abstract: Cognitive flexibility is a critical component of natural behavior, often impaired in neuropsychiatric disorders. When listening, we can choose whether to attend or ignore a sound source but the neural mechanisms involved in selective attention are unclear. Here we trained 5 ferrets to localise auditory (A, noise burst) or visual (V, light flash) stimuli that could occur at one of three locations. Trials could be A, V or AV. In the AV condition A and V stimuli were presented from separate locations and animals were cued by trial history to localise one modality while ignoring the other. Subjects performed extended blocks in which they localised either auditory or visual stimuli and reliably switched between A and V localisation over several test sessions.

During task performance, we simultaneously recorded multi-unit activity in primary auditory cortex (AC) and frontal cortex (FC) of two ferrets. AC units responded more strongly to auditory than visual stimuli with rapid changes in firing at stimulus onset / offset whereas FC units responded similarly across modalities with slower modulation in firing rate. To study the effects of attention, we compared population activity in FC and AC on correct and error trials when animals localized unisensory stimuli or attended to one modality in the AV compound. In AC we found little modulation of activity by attended modality but did observe changes in firing rate between correct and error trials during anticipatory activity in the 500 ms prior to stimulus onset. In FC we observed attentional modulation of baseline activity prior to stimulus onset and also following the animal's response. In each case, modulation was only observed on correct (and not error) trials. Furthermore the pattern of attentional effects was consistent with firing patterns for unisensory localization. For example, FC activity following behavioral response was greater for AV stimuli in attend-A than attend-V conditions, and also greater for A than V stimuli presented alone.

Our findings support a role for frontal cortex in maintaining representations of attended modality. In future our recordings offer the ability to study attentional modulation of functional connectivity between frontal and sensory cortices at both the level of both single unit activity and local field potentials.

Disclosures: **S.M. Town:** None. **J.K.A. Nordmark:** None. **J.K. Bizley:** None.

Poster

620. Spatial Factors of Crossmodal Integration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 620.12/RR10

Topic: D.09. Multisensory Integration

Support: NSERC #RGPIN-05435-2014

Network in Aging Research: Emergine Scholar Grant

Title: Multisensory integration using an aging brain

Authors: *M. BARNETT-COWAN;
Dept. of Kinesiology, Univ. of Waterloo, Waterloo, ON, Canada

Abstract: There is a long history of using psychophysical techniques to quantify, understand and treat changes in unisensory function that occur during the aging process. While it is well understood that sensory acuity declines with age, how the central nervous systems continues to integrate multisensory cues with unreliable sensory information available in later life is less clear. Perceptual binding of multisensory events occurs within a limited time span known as the temporal binding window. It has been shown that the ability to discriminate simultaneity, temporal order, and causal relationships among stimuli can become increasingly difficult as we age. Failure to correctly identify whether multisensory events occur simultaneously, what their temporal order is, or whether they should be causally bound can lead to inaccurate representations of the physical world, poor decision-making, and dangerous behaviour. Here I review work in our lab that has shown that i) older adults have an extended temporal binding window compared to younger adults, but which is task-specific, ii) older adults are less aware of the perceived onset of a fall compared to younger adults, and iii) the representation of the duration of multisensory events during a fall is distorted during a fall, particularly for older adults. These results and future work will be discussed in the context of developing new falls prevention assessment techniques to help prevent falls in later life.

Disclosures: M. Barnett-Cowan: None.

Poster

620. Spatial Factors of Crossmodal Integration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 620.13/RR11

Topic: D.09. Multisensory Integration

Title: One bout of open skill exercise improves multisensory perception in older adults

Authors: *A. SETTI[1], J. M. O'BRIEN[1], G. OTTOBONI[2], A. TESSARI[2];
[1]Sch. of Applied Psychology, Univ. Col. Cork, Cork, Ireland; [2]Dept. of Psychology, Univ. of Bologna, Bologna, Italy

Abstract: Inefficient multisensory perception of temporally asynchronous stimuli characterises older age. The time frame during which proximal information is integrated becomes extended, with negative consequences. Older adults, and more so, fall prone older adults, show higher susceptibility to the Sound-induced Flash Illusion (SiFI) than young adults, i.e. the illusory perception of two flashes when one single flash is presented with two beeps. Neural correlates of susceptibility to the illusion are cross-sensory interactions in early visual areas (V1) and Superior Temporal cortex. Older fallers who improve their balance with a programme of exergame training also show decreased susceptibility to the illusion. The present study aimed at testing the SiFI in older adults who are functionally able and regularly exercise to compare them with a control group of relatively sedentary older adults. Specifically, we assessed whether one bout of exercise improved performance on the SiFI and whether habitual exercise was predictive of SiFI performance. Additionally, we tested whether Open skill exercisers (i.e. exercise requiring responding to unpredictable environments e.g. aerobics classes, tennis) would show more benefit than Closed skill exercisers (i.e. exercise in predictable environments, e.g. swimming, running) or controls (e.g. reading club), based on the hypothesis that Open skill exercise provides a more stimulating multisensory environment fostering rapid multisensory training and adaptation. Fifty community-dwelling participants (17 Open skill; 17 Closed skill; 16 controls) with a mean age of 69, were tested on the SiFI and the Digit Span Forward test (results not reported here) before and after one session of their regular exercise or control activity. A 3 way ANOVA conducted on perceptual sensitivity d′ scores with Group, Time and Stimulus Onset Asynchrony as factors revealed a Time × Group interaction. Only Open skill exercisers' sensitivity improved after exercise. Random permutation ANOVA confirmed this significant Time × Group interaction. Habitual exercise (MET-minute, International Physical Activity Questionnaire Short Form) was not predictive of performance after exercise nor did it correlate with overall SiFI sensitivity.
In conclusion, Open skill exercisers were the only group showing immediate benefits, however no correlation with day-to-day exercise was found. This partially supports the hypothesis that both arousal and exercise mode contribute to perceptual sensitivity modulation in ageing. Beta

band activity, which evidence has shown to be modulated by exercise and is associated with SiFI, could underlie these effects.

Disclosures: **A. Setti:** None. **J.M. O'Brien:** None. **G. Ottoboni:** None. **A. Tessari:** None.

Poster

620. Spatial Factors of Crossmodal Integration

Location: Halls B-H

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Program#/Poster#: 620.14/RR12

Topic: D.09. Multisensory Integration

Support: NIDCD R01 DC013580

NIDCD K23 DC011298

Title: Integration of human visual and vestibular heading direction during eccentric viewing conditions.

Authors: *B. T. CRANE;
Otolaryngology, Univ. of Rochester, Rochester, NY

Abstract: Visual heading perception is strongly eye position dependent while vestibular or inertial headings are not. This study examined visual and inertial heading integration with eccentric eye positions in 5 humans. Experiments had randomly interleaved trials in which eye position was centered, 20° left, or right. A fixation was monitored using a binocular video eye tracking. The fixation point disappeared during the stimulus and eye position was maintained at the location. The stimulus was 2s of visual motion through a star field or analogous inertial motion. After each stimulus, subjects reported whether they felt each stimulus was consistent with motion left or right of the midline. In the visual only condition with eccentric viewing positions the average point of subjective equality (PSE) in which motion was equally likely to be perceived as left or right of center was 10° in the direction of gaze. Thus, visual heading perception was shifted in the direction opposite gaze, a shift consistent with retinatopic coordinates. When the experiment was conducted with an inertial stimulus in darkness, eccentric viewing positions had a smaller and opposite effect than they had with visual stimuli. The PSE with inertial stimuli in darkness was 5° in the opposite direction of gaze. Since eye position had opposite effects on the perceived directions of visual and inertial headings, provided a novel method of studying visual-vestibular multisensory integration that does not require the stimuli to be artificially offset from each other. Visual and inertial integration was studied by varying the coherence of the visual stimulus. When a 90% visual stimulus was used with an concurrent

inertial stimulus the PSE was shifted 7° in the direction of gaze, which dropped to 3° for 50% coherence, and -3° at 35%. Thus decreased visual coherence caused the heading perception to shift towards what was observed with a purely inertial stimulus. These findings were consistent with a Bayesian integration at low visual coherence but suggested closer to ideal performance that Bayesian predictions at high visual coherence.

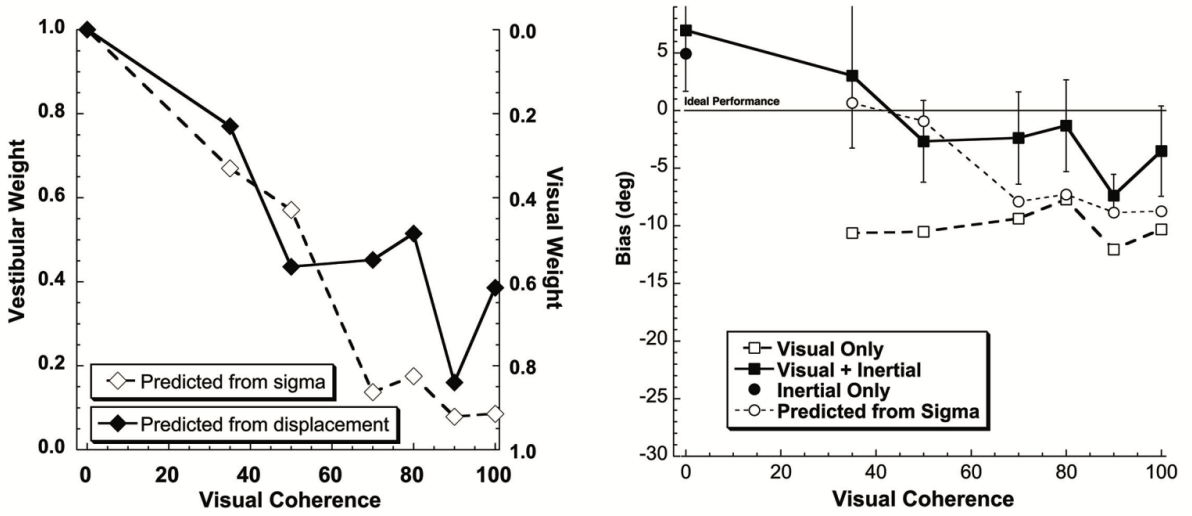

Figure. The left panel shows the predicted weights of the vestibular and visual cues based on the sigma or width of the psychometric function (open symptoms) and biases or displacement of the mean of the psychometric function (filled symbols). For high visual coherence the displacement predicts a higher vestibular weight than would be suggested by the sigma. The right panel plots the obserserved baises of the visual condition (open squares), inertial condition (filled circle) and combined (filled squares). The predictions of Bayesian integration based on the relative reliability of each (as determined by sigma) is shown with open circles. The observed responses (filled squares) are closer to ideal performance for high coherence visual stimuli that Bayesian integration suggests.

Disclosures: B.T. Crane: None.

Poster

620. Spatial Factors of Crossmodal Integration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 620.15/RR13

Topic: D.09. Multisensory Integration

Support: NIH Grant CA183492

Title: Age-related changes in multisensory temporal acuity and links to cognition.

Authors: *M. T. WALLACE[1], S. H. BAUM[2], R. A. STEVENSON[3];
[1]Hearing and Speech Sci., [2]Vanderbilt Brain Inst., Vanderbilt Univ., Nashville, TN; [3]Psychology, Univ. of Western Ontario, London, ON, Canada

Abstract: Background: In order to integrate sensory information across modalities into coherent, unified percepts, our nervous systems rely on the timing of sensory inputs. The temporal fidelity of sensory processing, however, declines with age, which has been hypothesized to result in decreased multisensory integration in older adult. Furthermore, sensory perception is the building block of myriad cognitive functions, and impairments in sensory processing, including those associated with healthy aging, can subsequently impact those cognitive functions.
Methods: To test the impacts of decreased temporal precision associated with healthy aging, we ran participants (18-79 years old, N = 48 with data collection ongoing) through a, extensive battery of perceptual and cognitive tests. First, participants' multisensory temporal fidelity was tested with a simultaneity judgment task used to extract each individual's "temporal binding window" (TBW), or the window of time within which an auditory and visual stimulus are bound into a single unified percept. As a measure of audiovisual integration, the well-characterized McGurk Effect was used, in which participants integrate a visual "ga" with an auditory "ba" to perceive the syllable "da." Finally, a number of cognitive tests were conducted, including the Buschke Selective Reminding Task, Choice Reaction Time, Critical Flicker Fusion, and Connor's Continuous Performance Task.
Results: Older adults showed significant decreases in multisensory temporal fidelity, indexed by wider TBWs. Additionally, older adults showed decreases in multisensory integration, as indexed by the McGurk effect. Importantly, correlations showed that, in older adults, decreases in temporal fidelity were correlated with decreases in multisensory integration ($p = 0.02$, $r=0.41$). Within older adults (45-70 years old), hierarchical clustering analysis demonstrated links between these perceptual processes and higher-order cognitive measures. For example, audiovisual temporal processing was linked to performance on the Selective Reminding Task.
Implications: These results suggest that changes in multisensory temporal fidelity commonly observed in healthy aging can have downstream impacts not only on perceptual abilities such as multisensory integration of speech signals, but also on higher-order cognitive processes.

Disclosures: **M.T. Wallace:** None. **S.H. Baum:** None. **R.A. Stevenson:** None.

Poster

621. Multi-Sensory Integration: Crossmodal Processing in Humans II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 621.01/RR14

Topic: D.09. Multisensory Integration

Support: NIH Grant R21DC013915

Title: Event-related potentials associated with somatosensory interaction with audio-visual speech processing

Authors: *T. ITO[1,2,3], H. OHASHI[2], E. MONTAS[2], V. L. GRACCO[2,4];
[1]Gipsa Lab, CNRS, St Martin D Heres Cedex, France; [2]Haskins Labs., New Haven, CT; [3]Univ. of Grenoble Alpes, Grenoble, France; [4]McGill Univ., Montreal, QC, Canada

Abstract: Speech perception is an interactive process involving both the auditory and visual modalities. Recent studies demonstrate that somatosensory input can also influence the process of auditory speech perception (Ito et al. 2009; Gick and Derrick 2009). However, it is unknown whether and to what extent somatosensory inputs modulate audio-visual speech perception. To address this question, we explored the neural consequence of somatosensory interactions with audio-visual speech processing under audio-visual speech processing conditions. Specifically, using the McGurk effect (the perceptual illusion that occurs when the auditory component of one sound is paired with the visual component of another sound, leading to the perception of a third sound) we assessed whether somatosensory orofacial stimulation influenced event-related potentials (ERPs) during audio-visual speech perception. We recorded ERPs from 64 scalp sites in response to audio-visual speech processing and somatosensory stimulation. We applied an auditory stimulus /ba/ synchronized with the video of congruent facial motion (the production of /ba/) or incongruent facial motion (the production of the /da/: McGurk condition). The congruent and incongruent audio-visual stimulations were randomly presented with and without somatosensory stimulation associated with facial skin deformation. We recorded ERPs under auditory alone, somatosensory alone, and auditory-somatosensory stimulus conditions. The subjects were asked to judge whether the production was /ba/ or not. We observed a clear McGurk effect in the behavioral responses with the subjects identifying the sound as /ba/ in the congruent audio-visual condition, but not in the incongruent condition. Concurrent somatosensory stimulation modified the ability of participants to correctly identify the production as /ba/ relative to the non-somatosensory condition. We found ERPs differences associated with the McGurk effect in the presence of the somatosensory conditions. ERPs for the McGurk effect reliably diverge around 280 ms after stimulation onset. The results demonstrate a clear multisensory convergence of somatosensory and audio-visual processing in both behavioral and neural processing and suggest that somatosensory information encoding facial motion also influences audio-visual speech processing.

Disclosures: **T. Ito:** None. **H. Ohashi:** None. **E. Montas:** None. **V.L. Gracco:** None.

Poster

621. Multi-Sensory Integration: Crossmodal Processing in Humans II

Location: Halls B-H

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Program#/Poster#: 621.02/RR15

Topic: D.09. Multisensory Integration

Support: NASA Nebraska Space Grant

Title: Stepping to injected colored rhythms effect gait entropy more for auditory stimuli than visual.

Authors: *J. FUJAN-HANSEN, T. J. RAND, M. MUKHERJEE;
Univ. of Nebraska At Omaha, Omaha, NE

Abstract: Introduction: Healthy adult systems rely upon a balance of sensory inputs to maintain strong posture and locomotion. This finely turned feedback is the result of contributions from our visual, auditory, vestibular and proprioceptive systems. This crucial balance is often altered as a result of pathology or extended time spent in space. Therefore, there is a direct need for techniques designed to discover effective locomotor restoration. The purpose of this study was to determine the degree to which injected colored rhythms can effect spatio-temporal gait patterns of healthy young adults via visual and auditory stimuli.
Methodology: This project utilized Virtual Reality technology along with auditory afferent feedback to affect spatio-temporal gait patterns in healthy young adults. Subjects participated in either visual or auditory groups. The former walked on a treadmill tied to optic flow presented via Virtual Reality technology. The optic flow was comprised of a simulated walkway which required subjects to make heel contact with their right foot with the incoming virtual tiles. The latter group also walked on a treadmill, but without any optic flow. They instead listened to an auditory stimulus to which they were instructed to match right heel strikes with the auditory beats. Both the visual and auditory stimuli were presented to the participants in three different noise structures: pink, white and periodic. Sample Entropy of spatio-temporal data was used to assess the ability of the individuals to flexibly alter the structure of their gait to match the sensory stimuli.
Results: Significance was found to exist in the spatial domain of step length during the auditory stimulus between both the pink and white noise structures ($p<0.045$), as well as the white and periodic ($p<0.046$). Differences within the temporal domain of stride time in the auditory stimulus did not reach significance. Significant differences were not found to exist in either the temporal nor spatial domains of the visual stimulus. Upon further analysis, half of the participants in the visual condition responded differently than the other half.
Conclusions: The results from this project illustrate that auditory stimuli with characteristic

temporal structures enable entrainment more than visual stimuli. This may be because visual stimuli demonstrate strong individualistic patterns.

Disclosures: **J. Fujan-Hansen:** None. **T.J. Rand:** None. **M. Mukherjee:** None.

Poster

621. Multi-Sensory Integration: Crossmodal Processing in Humans II

Location: Halls B-H

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Topic: D.09. Multisensory Integration

Support: DFG Grant KE1828/2-1

DFG Grant SE1859/3-1

ERC Grant ERC-2010-StG-20091209

German Federal Ministry of Education and Research 01GQ1416

Title: Prestimulus beta-band power predicts perception of the sound-induced flash illusion on a trial-by-trial basis

Authors: ***J. KEIL**, M. KAISER, J. BALZ, D. SENKOWSKI;
Charité Med. Sch., Berlin, Germany

Abstract: The sound-induced flash illusion (SIFI) is a well-known example for the influence of auditory information on visual perception. Presenting multiple auditory stimuli alongside a concurrent single visual stimulus can induce the illusory perception of multiple visual stimuli. Interestingly, the perception of the illusion fluctuates between trials, resulting in a bistable multisensory perception. Recent research identified a number of possible cortical mechanisms to explain the occurrence of the illusion. We, and others, have suggested that alpha-band power in visual cortical areas and beta-band power in the superior temporal gyrus predict the perception of the SIFI. Furthermore, neurotransmitter concentration in the latter cortical area significantly correlates with the SIFI perception. Moreover, it was recently proposed that the individual alpha-band frequency predicts the SIFI perception. These findings suggest an essential influence of the cortical state prior to stimulus onset on subsequent perception.
In the current experiment, we went beyond the broad dichotomy of illusion versus veridical perception. We assessed the relationship between oscillatory neural activity prior to stimulus onset and the subsequent perception on the single trial level. To this end, we recorded high-density EEG from 26 subjects and analyzed oscillatory neural activity between 5 and 40 Hz in

the 500 ms interval prior to stimulus onset. Subsequently, we computed a binomial regression between single-trial oscillatory neural activity and upcoming perception in each subject. Across subjects, we found that beta-band power and, to a lesser extent, alpha-band power predict the perception of the SIFI on a trial-by-trial basis. Our results thus corroborate previous findings on the crucial influence of the prestimulus cortical state, as reflected in oscillatory neural activity, on upcoming perception. Importantly, by showing a close link between oscillatory neural activity and perception on a trial-by-trial basis, the current results extend our knowledge on the relationships between cortical states and perception.

Disclosures: **J. Keil:** None. **M. Kaiser:** None. **J. Balz:** None. **D. Senkowski:** None.

Poster

621. Multi-Sensory Integration: Crossmodal Processing in Humans II

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Program#/Poster#: 621.04/RR17

Topic: D.09. Multisensory Integration

Support: German Federal Ministry of Education and Research 01GQ1416

German Research Foundation SE1859/3-1

Title: Prestimulus high gamma oscillations in the sensorimotor cortex predict response speed to visuotactile stimuli

Authors: *D. SENKOWSKI[1], M. KREBBER[1], J. KEIL[1], D. FRIEDMAN[2], P. DUGAN[2], W. DOYLE[3,4], O. DEVINSKY[2,4], E. HALGREN[5], T. THESEN[2];
[1]Charité, Univ. Med. Berlin, Berlin, Germany; [2]Dept. of Neurol., [3]Dept. of Neuro, [4]Dept. of Neurosurg., New York Univ. Sch. of Med., New York City, NY; [5]Multimodal Imaging Lab., Univ. of California, San Diego, CA

Abstract: Neural oscillations serve many purposes in the human brain, including the integration and routing of information across cortical areas. Previous research has also demonstrated the behavioral relevance of pre- and poststimulus neural oscillations in various frequency bands, such as delta, alpha, beta, and gamma oscillations. Thus far, it is not well understood how prestimulus high gamma power (> 80 Hz) relates to behavioral outcomes. In this intracranial EEG study we investigated how pre- and poststimulus gamma and high-gamma power in the sensorimotor cortex affects response speed to visuotactile target stimuli. Three epilepsy patients with subdurally implanted electrode arrays were presented with brief unisensory visual, unisensory tactile, or bisensory visuotactile stimuli via LEDs and solenoid tappers mounted to a

handheld foam cube. Alternating between blocks, individuals were instructed to respond either to visual or tactile inputs of unisensory or bisensory stimuli. For each individual, single-trial time-frequency resolved correlations between oscillatory power and response times were calculated using permutation tests with cluster-based correction for multiple comparisons. In all patients, increased prestimulus gamma power (about 60-140 Hz) in the sensorimotor cortex contralateral to the stimulated hand predicted longer reaction times to visuotactile stimuli when attention was directed to the visual modality. Prestimulus gamma power did not consistently correlate with the behavioral responses when paying attention to tactile stimuli. When analyzing the relationships between poststimulus power and response times a more heterogeneous pattern of positive and negative relationships across electrode sites emerged. Interestingly, the patterns of relationships between poststimulus power and behavioral responses were similar for the two attention conditions. We conclude that prestimulus gamma and high-gamma power in the sensorimotor cortex impedes responses to visual inputs of visuotactile target stimuli. Our study provides evidence for the behavioral relevance of prestimulus high gamma power in the processing of multisensory visuotactile stimuli.

Disclosures: **D. Senkowski:** None. **M. Krebber:** None. **J. Keil:** None. **D. Friedman:** None. **P. Dugan:** None. **W. Doyle:** None. **O. Devinsky:** None. **E. Halgren:** None. **T. Thesen:** None.

Poster

621. Multi-Sensory Integration: Crossmodal Processing in Humans II

Location: Halls B-H

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Program#/Poster#: 621.05/RR18

Topic: D.09. Multisensory Integration

Support: ERC-2009-AdG 249425-CriticalBrainChanges

DFG BA5600 1-1

Title: Adapting to visual and auditory low frequency modulated stimuli induced enhanced tactile frequency discrimination

Authors: ***S. BADDE**[1], L. THOMASCHEWSKI[2], H. STOFFREGEN[2], B. RÖDER[3]; [1]Dept. of Psychology, New York Univ., New York, NY; [3]Biol. Psychology and Neuropsychology, [2]Univ. of Hamburg, Hamburg, Germany

Abstract: Human perception includes redundant information across multiple sensory systems, for example, when recognizing objects. The present study tested whether tactile vibration frequency processing assesses overlapping neural systems with those involved in sound and

visual frequency processing. We measured tactile discrimination thresholds for vibrating stimuli (either 25, 30 or 200Hz) in a 2IFC task. Each trial was preceded by an adaptation phase in which either nothing (baseline), a flickering checkerboard (visual adaptation) or a sine tone (auditory adaptation) was presented for 12 seconds. Adapting stimuli always had a frequency of 25 or 30Hz. Tactile frequency discrimination performance within the same frequency range as the adapting stimulus was improved both in visual and in auditory conditions compared to the baseline condition. In contrast, tactile frequency discrimination performance for the 200Hz standard did not significantly differ between conditions, demonstrating frequency specificity of the adaptation effect. Adapting to frequency modulated visually and auditory stimuli induced frequency specific changes in tactile frequency discrimination suggesting overlapping neural representations of frequency across tactile, auditory, and visual modalities.

Disclosures: S. Badde: None. **L. Thomaschewski:** None. **H. Stoffregen:** None. **B. Röder:** None.

Poster

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Topic: D.09. Multisensory Integration

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Title: Intra- and multisensory interactions in congenitally deaf CI users

Authors: *I. FENGLER, D. BOTTARI, S. SOURAV, A. K. VILLWOCK, B. RÖDER;
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Abstract: Event-related potentials (ERP) are attenuated if the eliciting event was preceded by another event processed in overlapping neural circuits. These refractory effects (RFE) have been observed within and across sensory systems and can be used to assess intra- and multisensory processing capacities. Indeed, previous work has demonstrated quicker recovery of visual ERPs in congenitally deaf individuals, which was interpreted as evidence for compensatory changes within the visual system. In the present study, we assessed auditory, visual, and crossmodal RFE in a group of congenitally deaf recipients of a cochlear implant (CI). We expected larger crossmodal RFE for auditory stimuli following a visual stimulus but a lack of or reduced crossmodal RFE for visual stimuli following an auditory stimulus, which would indicate persisting changes in the crossmodal balance of cortical processing. Brief (50 ms) white noise

bursts and light flashes were used as standard stimuli. They were presented with an inter-stimulus interval (ISI) of either 1,000 or 2,000 ms. Mean amplitudes of auditory and visual ERP recorded from midline electrodes were analyzed as a function of group, electrode site, preceding ISI (short vs. long), and modality of the preceding stimulus (same vs. different). Both CI users and controls displayed unimodal and crossmodal RFE for auditory as well as visual ERP. These results suggest a recovery of multisensory interactions between the auditory and visual system despite transient auditory deprivation from birth.

Disclosures: I. Fengler: None. **D. Bottari:** None. **S. Sourav:** None. **A.K. Villwock:** None. **B. Röder:** None.

Poster

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Outstanding doctoral dissertation cultivation plan of action at ECNU (PY2015043)

Title: The causal role of the dorsolateral prefrontal cortex and somatosensory cortex in tactile-visual cross-modal working memory

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Abstract: The neural activity in the dorsolateral prefrontal cortex (DLPFC) has been associated with information integration across sensory modalities. Connections between the DLPFC and sensory cortices of different sensory modalities subserve the integrative function of the DLPFC. However, the causal role of the DLPFC in cross-modal working memory (WM) has not yet been established, and it is also unclear how top-down signals from the DLPFC influence activity of bilateral primary somatosensory cortices (SIs) in tactile-visual cross-modal WM. In the present

study, we applied single-*pulse trans*cranial magnetic stimulation (sp-TMS) to certain cortical areas of human participants at various time points, while they performed a tactile-visual delayed matching-to-sample task with a 2-second delay. sp-TMS over the contralateral DLPFC and contralateral SI at both an early sensory stage, 100ms after the onset of vibrotactile stimulation (200-ms duration), and an early maintenance stage, 300ms after the onset, significantly impaired the accuracy of task performance, and sp-TMS over the contralateral DLPFC and ipsilateral SI at a late maintenance stage (1600ms and 1900ms) also significantly disrupted the accuracy. Furthermore, significant correlations were observed between deteriorating effects of sp-TMS at 300ms over the contralateral SI and contralateral DLPFC on task performance. Taken together, these results indicate that the DLPFC plays an essential part in tactile-visual cross-modal WM, and cooperates with the contralateral SI in the early delay, and with the ipsilateral SI in the late delay respectively.

Disclosures: D. Zhao: None. **Y. Ku:** None.

Poster

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Topic: D.09. Multisensory Integration

Title: Modification of the central state of excitability using sustained electrical stimulation of the posterior roots in spinal cord injured humans

Authors: *M. R. DIMITRIJEVIC[1,2], M. KRENN[3,4], R. COBELJIC[5], E. SRNDIC[3], K. RIBARIC[6], W. MAYR[3];
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Abstract: The posttraumatic chronic condition of the spinal cord usually results in a variety of impaired movement and alteration of muscle tone. In this study, we describe modification of the central state of excitability of the spinal cord below the level of lesion. Therefore, we conditioned the lumbosacral spinal network using transcutaneous electrical stimulation of the posterior roots. For activation of the lumbosacral segments, we used for electrical stimulation surface electrodes on the T11-T12 vertebrae referenced to large paraumbilical electrodes. The sustained external input was applied using a “low” (5 - 50 Hz) or “high” (50 - 100 Hz) stimulation rate.
In five subjects (1 female) with chronic, traumatic, motor complete spinal cord injury we applied

a conditioning paradigm. We used two different test inputs for analyzing the modification of the central state of excitability. First, externally evoked posterior root reflexes (PRR) elicited by transcutaneous spinal cord stimulation and passive multi-joint hip and knee flexion for “low” and “high” stimulation rates, respectively.
In the major leg muscles, the motor output was recorded electromyographically by surface electrodes. In the case of “low” frequency stimulation, the analysis reveals a facilitation or a suppression of PRR responses in respect of different rates of the applied conditioning stimulation. Nevertheless, the effect on modification of the central state of excitability can also vary within different muscle groups of the same subject. For sustained stimulation rates higher than 50 Hz, a clear suppression of the motor output can be observed in subjects with increased central state of excitability during a passive motor task. In this cases, the activation of the leg muscles is nearly complete reduced.
The preliminary results suggest a projection of sensory axons to dorsoventral network that can be influenced by different range of stimulation rates. Finally, this study enables a methodology for understanding of the pathophysiology of spinal cord injury and restoration of functional movement.

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Poster

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VIEP-BUAP 174101

Catedra Marcos Moshinsky

VIEP-BUAP MEBI-EDH-16

Title: Internal multisensory stochastic resonance in visual and auditory pathways

Authors: *I. MENDEZ-BALBUENA[1], P. ARRIETA[2], N. HUIDOBRO[3], E. MANJARREZ[3]; [1]Facultad de Psicologia, Puebla, Mexico; [2]Facultad de Medicina, [3]Inst. de Fisiologia, Benemerita Univ. Autonoma de Puebla, Puebla, Mexico

Abstract: The aim of the present study was to demonstrate the electrophysiological occurrence of the internal multisensory stochastic resonance in the visual and auditory sensory modalities. We performed two experimental conditions (EC) in eight subjects; i.e., we applied, to both left and right eyes and ears: continuous visual noise of constant intensity versus continuous auditory noise of different amplitude levels (EC1), as well as auditory noise of constant intensity versus continuous visual noise of different amplitude levels (EC2). We examined whether a particular modulated Gaussian noise level can improve the EEG coherence. To quantify the EEG coherence we measured the area under the coherence curve above the significance level. The frequency window was 1-45 Hz. In order to obtain a general measure of the change of EEG synchronization in a particular level of noise, for each of the 30 electrodes we summed up all 29 possible combinations of pairwise coherence. For each noise level we subtracted the corresponding value to zero noise (ZN). We defined this number as the global weighted coherence relative to ZN (GRWC). We found that the GRWC values for all the subjects exhibited and inverted U-like function of the noise intensity. We compared the GRWC between low noise (LN), optimal noise (ON) and high noise (HN). The mean percentage of change in EC1, between LN and ON, was 1347.2 ± 578.2 % (mean ± SE), and in EC2 was 371.9 ± 139.5 % (mean ± SE). We performed the nonparametric Friedman test to examine the statistical significance of the change in the GRWC, between LN, ON and HN conditions. For EC1, the results showed significant differences between the three conditions: ($Ch2\ (2)=12.2$, $p<0.002$). The post hoc Wilcoxon test revealed statistically significant differences between LN and ON ($p<0.004$) and between ON and HN ($p<0.004$). For EC2, the results also showed significant differences between the three conditions: ($Ch2\ (2)=13$, $p<0.002$). The post hoc Wilcoxon test revealed statistically significant differences between LN and ON ($p<0.008$) and between ON and HN ($p<0.006$). In contrast, in both EC, no statistically significant differences were found between LN and HN conditions. Our electrophysiological study extends the psychophysical experiments by Manjarrez et al., (2007) in the context of an EEG paradigm. Furthermore it shows that the EC1 is more effective to produce a higher GRWC than the EC2. Our results demonstrate that visual and auditory noise could potentially modulate the EEG coherence amplitude by following the principles of the multisensory stochastic resonance phenomenon.

Disclosures: I. Mendez-Balbuena: None. **P. Arrieta:** None. **N. Huidobro:** None. **E. Manjarrez:** None.

Poster

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Topic: D.09. Multisensory Integration

Support: Qdai-jump Research Program, Grant Number 27818

Kobayashi International Scholarship Foundation

Title: Effect of an olfactory stimulus on color perception

Authors: *K. TAMURA[1], T. OKAMOTO[1,2];
[1]Fac. of Arts and Sci., [2]Grad. Sch. of Systems Life Sci., Kyushu Univ., Fukuoka, Japan

Abstract: Olfactory perception is known to be altered by observing different colors. Conversely, few studies have investigated whether visual perception is influenced by olfactory inputs. As color perception is generally less stable than other types of visual perception, we hypothesized that the perception of specific colors would be enhanced by olfactory input. To test this hypothesis, we analyzed event-related potentials (ERPs) in the brains of subjects presented with different color-odor combinations. Twenty volunteers (ten females) with normal color vision were tested under two conditions, control and odorant, the order of which was randomized. In the odorant condition, 0.1% decanal (vol/vol)—perceived as a citrus-like smell and associated with orange colors by 7/12 students—was volatilized in the experimental room. In both conditions, participants were required to state what color was presented in the preceding 1.5 s. Each participant viewed four types of color groups (orange, green, blue, and pink), each consisting of five color phases equally spaced by 10 degrees of hue angle, as specified in CIELAB color space. The ERPs of each participant were recorded using electroencephalography throughout the experiment. A positive peak was observed around 300 ms (p300-like component), just after the participants started to report the color. The mean amplitude of the p300-like component measured under the odorant condition was significantly smaller than that obtained in the control condition. In addition, within the odorant condition, the mean amplitude was significantly smaller in response to the orange group compared to the other color groups. Contrary to our hypothesis, the results indicate that the citrus-like smell suppressed the p300-like component in response to the orange group. We suggest that cross-modal interactions between visual and olfactory senses can influence (occasionally decrease) the perception of specific colors that are co-presented with a smell.
This work was supported by the Qdai-jump Research Program, Grant Number 27818, and the Kobayashi International Scholarship Foundation.

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Poster

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Title: Visuo-tactile motion processing in congenitally deaf humans

Authors: *A. K. VILLWOCK, D. BOTTARI, B. RÖDER;
Univ. of Hamburg, Hamburg, Germany

Abstract: Sensory deprivation has been shown to partially have a strong impact on the development of the spared modalities. However, to date, little is known about the impact of auditory deprivation on the development of multisensory processing. In the present study, we investigated the consequences of congenital deafness on the processing of visuo-tactile motion stimuli. To this aim, we recorded the event-related potentials (ERPs) in congenitally deaf signers (N = 21) and matched hearing controls during a visuo-tactile motion discrimination task. The visual and tactile stimulators were either moving congruently, that is, both moving upwards or downwards, or incongruently, that is, one stimulus moving upwards, the other downwards. The motion of standard stimuli was continuous, while deviants included an interrupted motion in one of the two modalities. Participants had to respond to rare deviants in both modalities, but only to those going into a specific direction. We expected an enhanced behavioral performance in the deaf group compared to hearing controls. Furthermore, we hypothesized to find a more anterior distribution of the ERPs in the deaf group compared to the hearing group after the visuo-tactile stimulation, indicating crossmodal plasticity. Deaf individuals committed more false alarms in the incongruent motion condition. In the ERPs to standard stimuli, the deaf group displayed a more anteriorly distributed potential compared to the hearing controls at a time window around 150 ms after stimulus onset. Moreover, we observed a delayed effect of motion congruency in the ERP of the deaf group compared to the hearing control group. These results suggest crossmodal reorganization - namely, an enhanced responsiveness to dynamic visuo-tactile stimulation in auditory areas - and an altered timing of the multisensory processing of motion direction after congenital deafness.

Disclosures: **A.K. Villwock:** None. **D. Bottari:** None. **B. Röder:** None.

Poster

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Topic: D.09. Multisensory Integration

Support: James S. McDonnell Foundation

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Riksbankens Jubileumsfond

Title: Rapidly induced auditory plasticity by imagined visual stimuli

Authors: *C. C. BERGER, H. H. EHRSSON;
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Abstract: The spatial recalibration of auditory perception that follows exposure to synchronous but spatially discrepant audiovisual stimuli—the ventriloquism aftereffect—has become a classic demonstration of the plasticity of the auditory system. This recalibration in auditory perception is considered to be an adaptive response that results from the repeated translocation of auditory stimuli towards the spatially discrepant visual stimuli. Recent evidence suggests that visual mental imagery can also lead to a translocation of perceived auditory stimuli (Berger & Ehrsson, 2013, 2014). In light of these findings, we investigated whether imagined visual stimuli led to the same rapidly induced auditory plasticity as real visual stimuli in four separate psychophysics experiments (n = 96). In our main experiment, we examined whether imagining a visual stimulus (white disk; visual angle = 1.72°) at the same time as a white noise burst (50 ms; rise/fall time = 5 ms) repeatedly for 30 s exposure periods led to a significant shift in the participants' point of subjective equality (PSE) of spatially localized auditory stimuli during subsequent test phases. We found a significant leftward shift in the participants' PSEs during post-exposure phases when the participants imagined visual stimuli in the center of the screen and the auditory stimuli were simultaneously presented 8° to the left during the exposure phases (i.e., rightward adaptation); and we found a significant rightward shift in the participants' PSEs when the auditory stimulus was presented 8 ° to the right during the exposure phases (i.e., leftward adaptation), compared to a control condition in which imagined visual stimuli and real auditory stimuli were presented in the center [$F(2, 46) = 15.29$, $p < .001$, $\eta^2_G = .17$]. This result was consistent with a 'real' visual stimulus version of the experiment in which the visual stimuli were actually presented rather than imagined [$F(2, 46) = 26.76$, $p < .001$, $\eta^2_G = .25$], and a comparison of the effect between the real-stimulus and imagined stimulus experiments revealed that there was no significant difference in

the ventriloquism aftereffects for these experiments [$t(46) = .067$, $p = .504$]. Furthermore, a control experiment revealed that the visual imagery-induced aftereffect did not transfer across different sound types ($ps > .05$). This result was consistent with findings from an additional control experiment using real visual stimuli as well as findings from previous studies. These findings are the first to demonstrate the cross-modal plasticity of our perceptual systems induced by mental imagery.

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Poster

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Topic: D.09. Multisensory Integration

Support: NSERC

Title: Interaction of attentional and movement-related modulation of evoked potentials in the primary and secondary somatosensory cortices

Authors: *J. R. TENNANT, M. S. ADAMS, W. R. STAINES;
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Abstract: Previous research suggests that the prefrontal cortex contributes to attention-related disinhibition of movement-related gating in the primary somatosensory cortex. Brown et al. (2015) provided evidence for this phenomenon by demonstrating that continuous theta burst stimulation applied over the dorsolateral prefrontal cortex results in an impaired ability to disinhibit task-relevant somatosensory information. Currently however, the precise stages of processing for these attentional and movement-related modalities are not well defined. We hypothesized that when attention is directed away from the somatosensory modality or somatosensory feedback is not task-relevant, gating would be enhanced. Conversely, when attention is directed towards the somatosensory domain or somatosensory feedback is task-relevant, we predicted a release of gating would occur. Somatosensory evoked potentials (SEPs) were elicited via median nerve stimulation at the wrist, and measured using EEG over the contralateral somatosensory cortex in five experimental conditions. In a baseline condition (rest), SEPs were evoked while participants maintained a neutral wrist position with no specific instructions for directing their attention. A passive movement condition was included to assess task-irrelevant movement-related gating. In this condition, SEPs were recorded while participants' stimulated wrists were moved through a random pattern of flexion/extension

movements within a 60-degree range. In an active tracking condition, participants' stimulated wrists were similarly passively moved through a range of flexion and extension movements for 7 seconds. Participants were then required to actively mirror the movements with their opposite limb, facilitating a measure of task-relevant movement-related gating. In a counting condition, participants counted the total number of stimulations received at rest, requiring an attentional focus on the somatosensory modality without a motor task requirement. Conversely, in a visual condition, participants attended to and traced a visual waveform presented on a screen using a wrist movement device to control a cursor, while receiving stimulation. Preliminary results provide evidence for attentional and movement-related modulation of somatosensory afferents in both the primary (N20-P27) and secondary (P100) somatosensory cortices, consistent with our hypotheses. These findings will provide a greater understanding of the somatosensory gating process, and be used for the investigation of post-concussive alterations.
Brown et al. (2015). *Exp Brain Res, 233*(3), 927-936.

Disclosures: J.R. Tennant: None. **M.S. Adams:** None. **W.R. Staines:** None.

Poster

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Wings for Life Spinal Cord Research Foundation (WFL-AT-007/11)

Title: Patterns of spinal sensory motor outputs of the human lumbar spinal cord disconnected from brain control can be elicited by sensory inputs

Authors: *M. KRENN[1,2], W. MAYR[1], J. L. VARGAS-LUNA[3,4], I. PERSY[5], M. R. DIMITRIJEVIC[6,7];
[1]Med. Univ. of Vienna, Wien, Austria; [2]Vienna Univ. of Technol., Vienna, Austria; [3]Med. Technol. Ctr., Reykjavik Univ. - Landspitali Univ. Hosp., Reykjavik, Iceland; [4]Escuela de Ingenieria y Ciencias, Tecnologico de Monterrey, Monterrey, Mexico; [5]Ludwig Boltzmann Inst. of Electrical Stimulation and Physical Rehabil., Vienna, Austria; [6]Baylor Col. of Med., Houston, TX; [7]Fndn. for Movement Recovery, Oslo, Norway

Abstract: Sensory motor circuits in the spinal cord are constructed with a fine specificity that coordinates motor behavior. Analysis of the dorsoventral connectivity patterns in an animal

model showed that the projections of sensory axons to discrete dorsoventral domains of the spinal cord without regard for motor neuron subtype or the presence of motor neurons [1]. By implications, the clustering and dorsoventral settling position of motor neuron pools serve as a determinant of the pattern of sensory input specificity and thus motor coordination. The mechanisms that direct sensory connections with their motor neuron pattern remain unclear. In this study, patterns of spinal sensory-motor output initiated by epidural spinal cord stimulation of lumbar posterior human cord determinant of the patterns of sensory inputs rate and strength.
Six subjects (two females) with chronic, traumatic, motor complete spinal cord injury were studied. The injury level was between Th2 and Th6. The epidural spinal cord stimulation consisted of a cylindrically shaped electrode array and a pulse generator (Pisces-Quad electrode, Model 3487A and Itrel 3, Model 742, Medtronic Inc., Minneapolis MN, USA). Across subjects, the rostrocaudal positions of the electrodes ranged from vertebral levels Th11-L1. The motor output was recorded electromyographically in the major leg muscle. For analysis, the root mean square value (E_{RMS}) of the signal was calculated in a window of 3 s.
The E_{RMS} was facilitated in all muscle groups by 66.3% between a stimulation rate of 2.1 Hz and 10 Hz. Above 10 Hz, the modification of the motor output with increasing stimulation rates showed a progressive suppression. Therefore, the E_{RMS} declined around 19%, 51% and 66% for a stimulation rate of 16, 20 and 30 Hz, respectively. At a sustained stimulation of 100 Hz, the responses were nearly diminished.
In our human data, we have found crucial evidence of consistently reproducibly patterned response. In contrast, the animal research has different motor behavior, but it predicts some interesting cluster activity which we interpret in our subjects without variability. Moreover, if this pattern response is augmented or suppressed, we can demonstrate the same effect on the result of independent motor unit's activity from background activity.
Intriguing differences between results and the insights gathered gives us the opportunity to discuss the significance of the patterned response modifying the motor control in spinal cord injury subjects. 1. Sürmeli G. et al. 2011, Cell, 147(3):653-65

Disclosures: **M. Krenn:** None. **W. Mayr:** None. **J.L. Vargas-Luna:** None. **I. Persy:** None. **M.R. Dimitrijevic:** None.

Poster

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Title: Visual selectivity for mouth movements predicts auditory response in human superior temporal sulcus

Authors: *L. L. ZHU, M. S. BEAUCHAMP;
Baylor Col. of Med., Houston, TX

Abstract: Speech perception is a multisensory process entailing the use of both visual information from the talker's face and auditory information from the talker's voice. Posterior temporal cortex, comprising superior temporal sulcus and superior temporal gyrus (pSTS/STG), is thought to be a key brain locus for the integration of visual and auditory speech information. However, we know relatively little about the neuroanatomical organization of this region that enables integration. Previous studies have shown that sub-regions of the pSTS/STG show preferential responses to different categories of visual stimuli and different categories of auditory stimuli, but little is known about the relationship between these different axes of selectivity. In natural environments, we usually see visual mouth movements at the same time as we hear vocal speech. This association in environment statistics could have neural consequences: we hypothesized that regions of the pSTS/STG with a preference for *visual* mouth movements should respond strongly to *auditory* stimuli, especially speech. As a control, we examined regions of the pSTS/STG that previous studies have shown are selective for visual eye movements. To test this hypothesis, we used blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI) to scan twenty subjects. In the first experiment, we mapped voxels by their visual preference and measured the response to speech stimuli consisting of auditory recordings of short stories. In pSTS/STG, visual mouth-movement preferring voxels showed greater responses to auditory speech than eye-movement preferring voxels (LH: 1.6±0.2% vs. 0.1±0.1%, $p<8.6x10^{-5}$; RH: 1.2±0.2% vs. -0.2±0.1%, $p<4.0x10^{-6}$). In the second experiment, we compared the response of visual mouth-movement preferring voxels to auditory vocal vs. non-vocal sounds. Mouth-movement preferring voxels showed greater response to vocal than non-vocal sounds (1.2±0.1% vs. 0.5±0.1%, $p=5.9x10^{-6}$). Our study demonstrates that subregions of the pSTS/STG respond strongly to both visually-presented mouth movements and auditory speech, suggesting that these stimulus features are coded together in small populations of neurons.

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Poster

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Location: Halls B-H

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Program#/Poster#: 621.16/SS7

Topic: D.09. Multisensory Integration

Support: Grant-in-Aid for Scientific Research (A) (#25249026) and (B) (#25303013)

Title: Cross-modal interactions of haptic-visual roughness matching in the bilateral fusiform gyrus

Authors: *J. YANG[1], Y. YU[1], H. SHIGEMASU[2], H. KADOTA[2], K. NAKAHARA[2], Y. EJIMA[1], J. WU[1];
[1]Okayama Univerisity, Okayama, Japan; [2]Kochi Univ. of Technol., Kochi, Japan

Abstract: Humans effortlessly distinguish numerous different categories of material (such as a stone, metal, wood etc.) at a glance and can recognize many specific somatosensory feelings at the same time. This suggests that information about an object is built by different sensory modalities that converge somewhere in the human brain to form representations that are invariant to the input modality. The fusiform gyrus (FG) is located between the lingual gyrus and parahippocampal gyrus and is primarily involved in the higher function of visual texture perception. However, recent neuroimaging studies suggest that the FG represents textures in a way that reflects not only visual but also non-visual object properties, such as haptic roughness. Therefore, the FG is considered a multisensory hub that plays an important role in haptic-visual texture integration/interaction processing. In the present study, we therefore used the modified delayed-match-to-sample paradigm to reveal FG activations related to cross-modal interactions of haptic-visual roughness matching. Eighteen healthy subjects were asked to remember the roughness of the first stimulus (visual or haptic) during an encoding phase and to identify a stimulus that was the same or was closely related in roughness to one of five stimuli (visual or haptic) during the recognition phase. In essence, the recognition phase consists of a recall of information about the roughness of the first stimuli, a comparison of the roughness of the first stimulus to the ongoing stimuli and a resolution of a final decision. Therefore, the activations of cross-modal tasks (HV, haptic-visual and VH, visual-haptic) compared to the corresponding unimodal tasks (VV, visual-visual and HH, haptic-haptic) will reveal cross-modal specific activations. The results demonstrated that HV>VV contrast revealed significant activations in the bilateral FG that were not apparent in the VH>HH contrast. This finding suggests that the bilateral FG of humans mediates roughness information about objects in multimodalities. However, bilateral FG activation may be more important for roughness transformation from haptic to visual modalities compared to the opposite orientation.

Disclosures: **J. Yang:** None. **Y. Yu:** None. **H. Shigemasu:** None. **H. Kadota:** None. **K. Nakahara:** None. **Y. Ejima:** None. **J. Wu:** None.

Poster

621. Multi-Sensory Integration: Crossmodal Processing in Humans II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 621.17/SS8

Topic: D.09. Multisensory Integration

Title: Neural correlates of robot-controlled psychosis-like state: an eeg study in healthy subjects

Authors: *F. BERNASCONI[1], M. SOLCÀ[2], A. GUGGISBERG[3], G. ROGNINI[2], A. SERINO[2], O. BLANKE[2];
[1]Lab. of Cognitive Neurosci. (LNCO), [2]Ecole Polytechnique Fédérale De Lausanne (EPFL), Genève, Switzerland; [3]Univ. of Geneva, Geneva, Switzerland

Abstract: The feeling of a presence (FoP), the sensation that somebody is nearby when no one is actually present, is reported mainly by neurological and psychiatric patients but also by healthy individuals facing extreme situations. Recent findings demonstrated that sensorimotor conflicts between upper limb movements and somatosensory feedback on the back may induce FoP in healthy subjects, suggesting that FoP is caused by misperceiving the source and identity of sensorimotor signals of one's own body. Despite these new insights into the FoP, very little is known about its neural basis. Here we applied the same sensorimotor conflict in healthy volunteers, while brain activity was measured with a 64-channels EEG. We compared the oscillatory power and estimated the inter-site phase clustering by means of the weighted phase-lag index (wPLI), in response to the condition with a sensorimotor conflict with the condition without a sensory motor conflict. Our results indicate a modulation in the alpha frequency band (8-11Hz), over the left frontocentral sensors. The functional connectivity analyses indicated a desynchronization of the gamma frequency band (31-45Hz), between somatosensory areas and frontoparietal areas in the condition with sensorimotor conflict. Collectively, our results provide new insights into the neural correlates of the robotically induced psychotic-like state, and indicate the frontoparietal cortex as the critical network associated to it. Interestingly, the involvement of the frontoparietal areas is supported by previous finding from neurological FoP patients, which indicated that the FoP is associated with three distinct brain regions: temporoparietal, insular, and especially with the frontoparietal cortex.

Disclosures: **F. Bernasconi:** None. **M. Solcà:** None. **A. Guggisberg:** None. **G. Rognini:** None. **A. Serino:** None. **O. Blanke:** None.

Poster

621. Multi-Sensory Integration: Crossmodal Processing in Humans II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 621.18/SS9

Topic: D.09. Multisensory Integration

Support: NSERC Grant to W.R. Staines

Title: Attentional gating at early cortical processing stages is associated with changes in behavioural performance on a motor task with sensory conflict

Authors: *M. S. ADAMS, J. R. TENNANT, W. R. STAINES;
Univ. of Waterloo, Waterloo, ON, Canada

Abstract: While there is evidence to show early modulation of modality-specific somatosensory cortical potentials when two stimuli are task-relevant, less is understood about the cortical and behavioural correlates of early modality-specific sensory gating. This study examined attention-related changes in early somatosensory and visual processing and the effect of an unattended sensory stimulus on cortical processing and behavioural performance. For Part 1, it was hypothesized that early visual and tactile cortical responses would be diminished when unattended; that early cortical responses to task-relevant stimuli would be unchanged in the presence of a cross-modal distractor stimulus; and that participants' ability to accurately grade the intensity of stimuli would be consistent whether they were presented alone or with a cross-modal distractor. For Part 2, it was hypothesized that continuous theta burst stimulation (cTBS) to the prefrontal cortex (PFC) would diminish the effect of the attentional gating, resulting in similar ERP amplitudes for attended and unattended stimuli.
Healthy participants underwent two experimental sessions. In the first, electroencephalography (EEG) was collected as participants performed a sensory selection task which required graded motor responses be made to the amplitudes of episodic visual and tactile stimuli presented individually or concurrently. Attention was randomly directed for each block to either visual or tactile stimuli, which were presented as single visual, tactile, or simultaneous cross-modal stimuli. Event-related potentials (ERPs) were timelocked to visual and tactile stimuli, creating attended and unattended stimulus conditions. In the second session, the task was repeated and EEG was collected pre- and post-cTBS to the PFC. The somatosensory N70 potential was significantly diminished when tactile stimuli were unattended. When visual stimuli were unattended, similar attenuation occurred later in the processing stream (visual P2). When unattended tactile stimuli acted as distractors, this early gating resulted in unchanged cortical responses to target stimuli and no decrease in grading accuracy. Conversely, since early visual gating was not observed, presenting these stimuli as unattended distractor resulted in smaller-amplitude cortical responses to attended stimuli and less accurate grading. This modality specific

gating was impaired following cTBS to the PFC. This study suggests that early gating of unattended stimuli supports modality-specific cortical processing of target stimuli as well as task performance. It also suggests a role for the PFC in early attentional gating.

Disclosures: **M.S. Adams:** None. **J.R. Tennant:** None. **W.R. Staines:** None.

Poster

621. Multi-Sensory Integration: Crossmodal Processing in Humans II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 621.19/SS10

Topic: D.09. Multisensory Integration

Support: NIH Grant R01 NS065395

Title: Converging evidence from ECoG and fMRI for an anterior to posterior distinction in the superior temporal gyrus for audiovisual speech processing

Authors: ***M. OZKER**[1], D. YOSHOR[2], M. BEAUCHAMP[2];
[1]UT Hlth. Sci. Ctr. At Houston, Houston, TX; [2]Baylor Col. of Med., Houston, TX

Abstract: During speech perception, visual information from the face of the talker can compensate for noisy auditory speech. We combined neural recordings from electrodes implanted in the brains of epileptic patients (electrocorticography or ECoG) and BOLD fMRI in normal subjects, to examine the neural mechanisms of multisensory speech perception. Subjects viewed audiovisual words with either clear auditory speech (AV) or noisy auditory speech (AnV), identifying the presented words with a button press. In ECoG, electrodes in superior temporal gyrus (STG) responded to words with a burst of high-gamma (70-100 Hz) activity. Electrodes in the anterior STG (n=16) showed more activity for AV speech while electrodes in the posterior STG (n=12) showed more high-gamma activity for AnV speech (*Anterior*: AV=175±40%, AnV=75±10%; *Posterior*: AV=80±20%, AnV=120±20%, mean±SEM, RM-ANOVA $p=10^{-5}$). To verify that the sharp boundary observed was not a result of abnormal brain organization in epileptic patients, we performed the same experiment with BOLD fMRI in healthy subjects. The same anterior-to-posterior boundary in responses was observed (*Anterior*: AV=0.32±0.02%, AnV=0.22±0.03%; *Posterior*: AV=0.27±0.03%, AnV=0.32±0.02%, $p=10^{-4}$). Adding noise to the auditory component of speech resulted in weaker responses in the anterior STG but not in the posterior STG, possibly because the posterior STG receives more visual inputs that compensate for the degraded auditory signals.

Disclosures: **M. Ozker:** None. **D. Yoshor:** None. **M. Beauchamp:** None.

Poster

621. Multi-Sensory Integration: Crossmodal Processing in Humans II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 621.20/SS11

Topic: D.09. Multisensory Integration

Support: ERC - 2009 - AdG249425 – CriticalBrainChanges

Title: Recovery of visual influence on auditory motion processing despite persisting cross-modal changes in sight-recovery individuals

Authors: *M. J. GUERREIRO, L. PUTZAR, B. RÖDER;
Univ. of Hamburg, Hamburg, Germany

Abstract: The extent to which atypical sensory experience early in life affects the subsequent development of multisensory interactions in humans remains largely unknown. In particular, although several studies have assessed the impact of a transient phase of congenital blindness on visual motion processing, no study has yet investigated its impact on multisensory interactions for motion processing. In the present study, we investigated this question by measuring visual and auditory motion aftereffects induced by adaptation to visual or auditory motion in a group of six cataract-reversal individuals (aged 16-43 years, M = 28.7, SD = 9.7, 4 females), in a group of 20 normally sighted controls (aged 15-63 years, *M* = 23.8, *SD* = 10.5, 10 females) and in a group of five visually impaired controls (aged 34-56 years, *M* = 43.0, *SD* = 7.9, 4 females). The results revealed no differences between groups in unisensory auditory motion aftereffects, in line with previous studies showing no differences in auditory cortical processing of auditory motion between sight-recovery individuals and normally sighted controls. By contrast, cataract-reversal individuals and visually impaired controls exhibited enhanced unisensory visual motion aftereffects, possibly indicating a higher visual cortical excitability in individuals who experienced some degree of visual deprivation. Most important, there were no significant differences between groups in visually-induced auditory motion aftereffects, indicating that visual motion signals were capable of influencing the perception of auditory motion in cataract-reversal individuals to a similar extent as in normally sighted controls and visually impaired controls. Yet, auditory-induced visual motion aftereffects occurred only in cataract-reversal individuals - but not in normally sighted controls or visually impaired controls -, indicating that only in individuals who lacked appropriate visual input early in life did auditory motion signals influence the perception of visual motion. In contrast to what has been reported for audio-visual speech perception, the present findings demonstrate a cross-modal influence of vision on audition for motion processing in individuals who recovered from a transient phase of congenital blindness. Additionally, only in congenital cataract-reversal individuals was visual motion processing influenced by auditory motion signals. Since this effect was motion direction specific,

we speculate about some possibly beneficial effects of cross-modal plasticity associated with the period of total blindness on visual recovery within the dorsal processing stream.

Disclosures: **M.J. Guerreiro:** None. **L. Putzar:** None. **B. Röder:** None.

Poster

621. Multi-Sensory Integration: Crossmodal Processing in Humans II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 621.21/SS12

Topic: D.09. Multisensory Integration

Support: DFG, Cluster of Excellence "Hearing4all" (EXC 1077/1)

Title: Age-related hearing loss modulates functional connectivity between auditory and visual cortex

Authors: *S. PUSCHMANN, C. M. THIEL;
Univ. of Oldenburg, Oldenburg, Germany

Abstract: Previous work provided compelling evidence that a long-lasting auditory sensory deprivation leads to a cross-modal reorganization of auditory cortex, resulting in increased neural responses to non-auditory sensory input and changes in functional and structural connectivity to other sensory cortices. Recent data however indicate that such cross-modal neuroplastic changes may not only occur following severe and early-onset hearing impairments but rather also following moderate degrees of age-related high-frequency hearing loss (presbycusis). We here used functional magnetic resonance imaging (fMRI) to investigate how such potential cross-modal changes affect the processing of audiovisual information in older hearing-impaired subjects.
Older participants (N = 20, 61 ± 5 years) with a varying degree of high frequency hearing loss (high frequency threshold range: 10 - 67 dB HL) performed an auditory stimulus categorization task, in which they had to categorize rising vs. falling frequency-modulated tones presented alone or in the context of matching or non-matching visual motion. A motion only condition served as control for a visual take-over of auditory cortex.
BOLD response amplitudes as well as functional connectivity between auditory and visual cortex during audiovisual processing and at rest were analyzed as a function of high-frequency hearing thresholds. While hearing loss did not affect BOLD responses to auditory, visual, or audiovisual stimuli per se, we observed a significant hearing loss-related increase in functional connectivity between auditory cortex and the motion-sensitive visual area MT when processing matching audiovisual input. Similarly, resting state connectivity between area MT and auditory cortex

increased with hearing loss, suggesting a permanent, task-independent change in coupling between visual and auditory sensory areas with age-related hearing loss.
Our data provide first evidence for hearing loss-related changes in cross-modal functional connectivity in presbycusis. In line with the view that older hearing-impaired listeners tend to make use of additional visual motion cues, like lip-movements, to ease auditory comprehension in challenging listening environments, hearing loss specifically modulated the connectivity between auditory cortex and area MT, which is involved in visual motion processing, but did not affect auditory cortex coupling to earlier visual areas. Our results add up on the increasing evidence suggesting that cross-modal neuroplastic changes in the auditory system do not only occur in severe hearing impairments with early onsets, but also develop as hearing abilities decline in age.

Disclosures: S. Puschmann: None. **C.M. Thiel:** None.

Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 622.01/SS13

Topic: E.02. Cerebellum

Support: Max Planck Society

Title: Mechanisms of Purkinje cell-dependent instructive signaling in the cerebellum

Authors: *A. BONNAN, J. RICHTER, C. A. BAKER, M. M. BOLTON, J. M. CHRISTIE; MPFI, Jupiter, FL

Abstract: The cerebellum is known to play a crucial role in motor learning. Purkinje cells (PC) give rise to the sole output of the cerebellar cortex and are therefore pivotal in this process. PCs can fire two different types of spikes: simple spikes (SS) in response to parallel fiber (PF) stimulation and complex spikes (CS) as a result of climbing fiber input. The CS is a multicomponent response that includes a burst of sodium spikes at the soma and a dendrite-wide Ca^{2+} transient. A classic theory of cerebellar function postulates that CFs carry instructive signals to PCs that guide learning. However, recent findings indicate that learning may occur independent of CF activity suggesting the existence of other candidate instructive signals. To study the mechanistic basis of instructive signaling, we used the vestibulo-ocular reflex (VOR), a compensatory eye movement in response to head motion that is subject to re-calibration through cerebellar-dependent plasticity. We used optogenetics to selectively manipulate PC activity during vestibular stimulation and probe for the consequences of this activity by examining the

gain of the VOR. Using subcellular targeting motifs to limit expression of channelrhodopsin to the PC soma, we were able to evoke activity either with or without dendritic Ca^{2+} influx during vestibular stimulation. We found that PC activity associated with dendritic Ca^{2+} was sufficient to induce a change in VOR gain after 30 min of pairing with vestibular stimulation. However learning did not occur when Purkinje cells were activated without a dendritic Ca^{2+} transient. Our results suggest that Ca^{2+} influx in PC dendrites is a key component for learning and point to PC dendrites as the initial site of plasticity as predicted by early theories.

Disclosures: A. Bonnan: None. **J. Richter:** None. **C.A. Baker:** None. **M.M. Bolton:** None. **J.M. Christie:** None.

Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 622.02/SS14

Topic: E.02. Cerebellum

Support: NIH Grant R01 NS072406

NIH Grant P30 NS069375

Title: Synaptic learning rules in the cerebellum vary with the functional requirements of the circuit

Authors: *A. SUVRATHAN, H. L. PAYNE, J. L. RAYMOND;
Neurobiology, Stanford Univ., Stanford Univ., Stanford, CA

Abstract: The cellular anatomy is highly uniform throughout the cerebellum, and it has been widely assumed that the synaptic physiology is also uniform. In contrast, we report that the rules governing the induction of plasticity at an anatomically-defined type of synapse vary considerably across different regions of the cerebellum, in a way that appears to be tuned to the behavioral function.
In vivo, cerebellar climbing fibers encode errors during motor learning, and a single spike in a climbing fiber triggers a decrease in Purkinje cell firing on the next trial (Medina & Lisberger, 2008). We found a candidate synaptic mechanism at the parallel fiber-to-Purkinje cell (pf-PC) synapses *in vitro,* where a single pairing of parallel fiber and climbing fiber stimulation induced a transient depression of pf-PC synaptic strength. We show that both *in vivo* and *in vitro,* this single-trial plasticity is narrowly tuned for a climbing fiber delay of 120 ms, which precisely matches the delay in climbing fiber responses to errors during oculomotor learning. Although the

relationship between single-trial and long-term depression (LTD) is not fully understood, we found that LTD at these synapses had the same, stringent timing requirements for induction as the single-trial plasticity.
The timing requirements for plasticity in the cerebellar flocculus are unlike those described previously anywhere in the brain. There is a narrow window for association (tens of milliseconds), as observed for spike timing dependent plasticity (STDP), but, unlike STDP, this window is offset from coincident by >100ms. The properties of LTD at the pf-PC synapses in the flocculus are also unlike what has been described previously in other regions of the cerebellum. In the vermis, for example, which supports a range of functions, the pf-PC synapses have been reported to undergo LTD in response to a broad range of climbing fiber delays. Our analysis of single-trial plasticity at pf-PC synapses in the vermis suggests that different cells are tuned for different climbing fiber delays, so that the population average appears broadly tuned.
Our results demonstrate that cerebellar synaptic plasticity at parallel fiber-to-Purkinje cells synapses does not follow uniform rules for induction, but varies across functionally distinct regions of the cerebellum, and that the rules in the flocculus are precisely tuned to the functional requirements of the oculomotor learning it supports.

Disclosures: **A. Suvrathan:** None. **H.L. Payne:** None. **J.L. Raymond:** None.

Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 622.03/SS15

Topic: E.02. Cerebellum

Support: MH 46904

MH 74006

Title: Cerebellar cortex mechanisms supporting extinction and reacquisition of conditioned eyelid responses at different inter-stimulus intervals.

Authors: *H. E. HALVERSON, A. KHILKEVICH, M. D. MAUK;
Ctr. for Learning and Memory, Univ. of Texas At Austin, Austin, TX

Abstract: The cerebellar cortex is necessary for expression and extinction of properly timed conditioned eyelid responses (CRs) at different inter-stimulus intervals (ISIs). Cortical involvement in expression of CRs is clear, the mechanisms within the cortex supporting subsequent extinction and reacquisition of CRs at different ISIs have not been described.

Relating changes in Purkinje cell (PC) and molecular layer interneuron (MLI) responses to the rate of extinction and reacquisition of CRs at different intervals is necessary to establish the cerebellar rules governing these processes. Time demands at different ISIs could lead to different interactions between PCs and MLIs and the rate of CR extinction observed at short and long intervals. Before training a 12 tetrode hyperdrive array was implanted dorsal to the ipsilateral (to the trained eye) anterior lobe. Neuronal activity was recorded during initial CR expression and extinction with delay conditioning using a 550 ms tone conditioned stimulus (CS) and 50 ms periorbital shock (2-3 mA) unconditioned stimulus (US). After initial training with a 500 ms ISI, shorter (ISI 250) and longer (ISI 750) intervals were used to investigate interactions between PC and MLI responses during extinction and reacquisition. Eyelid PCs were identified by the presence of short latency US-evoked complex spikes. Eyelid MLIs were identified by a two step process using baseline firing properties and correlation with eyelid CRs. Reacquisition at longer ISIs would often show CRs with onsets that matched previous training at shorter ISIs early within a session. These CRs influenced by previous short ISI training would extinguish during the session. Extinction of CRs at longer intervals (ISI 500 and above) was faster than at shorter ISIs. Activity of eyelid PCs and MLI tracked CRs closely during extinction at longer ISIs and were more variable at shorter ISIs. These results suggest that time demands on the cerebellum during extinction lead to increased variability between PCs, MLIs and the rate of extinction of eyelid CRs.

Disclosures: H.E. Halverson: None. **A. Khilkevich:** None. **M.D. Mauk:** None.

Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 622.04/SS16

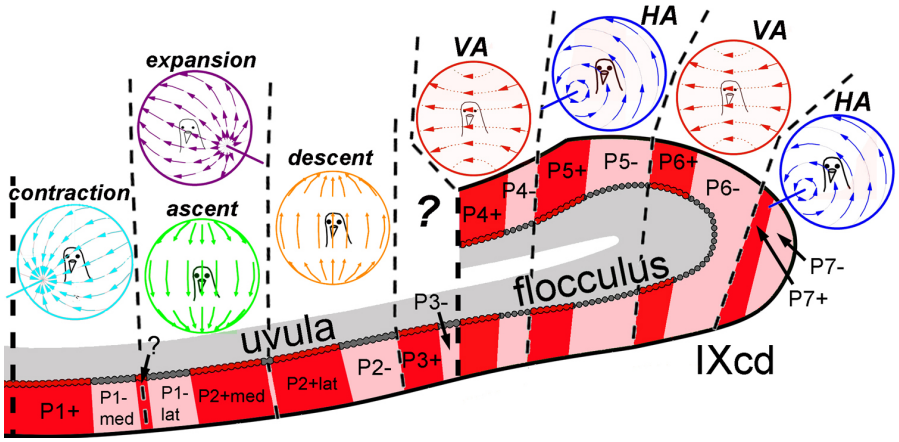
Topic: E.02. Cerebellum

Support: CIHR Grant 446013

Title: Zebrin positive and negative Purkinje cells in the vestibulocerebellum of pigeons differ with respect to the degree of modulation in response to optic flow stimuli.

Authors: *R. LONG[1], J. M. P. PAKAN[2], D. J. GRAHAM[3], D. R. WYLIE[4];
[1]Neurosci. and Mental Hlth. Inst., Univ. of Alberta, Edmonton, AB, Canada; [2]Ctr. for Integrative Physiol., Univ. of Edinburgh, Edinburgh, United Kingdom; [3]Lervig Aktiebryggeri, Stavanger, Norway; [4]Neurosci. and Mental Hlth. Inst., Edmonton, AB, Canada

Abstract: Zebrin II (ZII, the isoenzyme aldolase C) is heterogeneously expressed by cerebellar Purkinje cells (PCs), such that there are alternating sagittal stripes with high ZII immunoreactivity (ZII+) interdigitated with stripes where PCs show little or no ZII immunoreactivity (ZII-). Within the vestibulocerebellum (VbC) in pigeons (Columba livia), it has been shown that a ZII+/- stripe pair constitutes a functional unit, in so far as the complex spike activity (CSA) of all Purkinje cells in the stripe pair prefer the same type of optic flow stimuli (see Figure). In this report we show that ZII+ and ZII- Purkinje cells show differences in the depth of modulation to optic flow stimuli. We recorded the CSA of Purkinje cells in the VbC of anesthetized pigeons in response to large (90^o horizontal X 75^o vertical) drifting sine wave gratings moving in 8 different directions (45^o apart). For all recordings, the stimulus was centered in the frontal part of visual field, and the spatial and temporal frequency were kept constant (0.5cpd, 0.5Hz). At some recording sites we made a small injection of a fluorescent tracer. At the end of the experiment, the pigeons were perfused, the brains extracted, sectioned, and processed for ZII. In this way we determined the ZII signature of each CSA recording site. The recordings were analyzed offline using Spike2 and individual cells were isolated based on waveform shape and amplitude. Direction tuning curves were constructed using SigmaPlot. We found that the depth of modulation was greater for the ZII+ Purkinje cells ($p<0.001$): the CSA was greater to motion in the preferred direction, and there was greater inhibition to motion in the anti-preferred direction. We suggest that the difference in modulation is due to a feedback mechanism to the inferior olive from the ZII+ Purkinje cells. The ZII+ Purkinje cells tend to project to areas of the vestibular nuclei that in turn project to the areas of the inferior olive giving rise to climbing fibres to those Purkinje cells. The ZII- Purkinje cells tend to project to areas of the vestibular nuclei that do not project back to the inferior olive.



Disclosures: **R. Long:** None. **J.M.P. Pakan:** None. **D.J. Graham:** None. **D.R. Wylie:** None.

Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 622.05/SS17

Topic: E.02. Cerebellum

Support: Human Brain Project (HBP-604102)

Title: Two-photon imaging reveals long-term changes in cerebellar granule cell responsiveness following high-frequency mossy fibers stimulation

Authors: M. TOGNOLINA[1], L. MAPELLI[1], *E. D'ANGELO[1,2];
[1]Univ. of Pavia, 27100 Pavia, Italy; [2]Brain Connectivity Center, C. Mondino, Pavia, Italy

Abstract: The investigation of the spatiotemporal organization of neuronal activity in local microcircuits requires simultaneous and fast recordings from multiple neurons with a single-cell resolution. Although this can be obtained through optical techniques, a parallel signals detection requires the presence of multiple confocal excitation volumes that cannot be achieved through traditional confocal and two-photon microscopy. The use of a Spatial Light Modulator (SLM) to divide a coherent excitation light in multiple diffraction limited focal points, which can be configured in different patterns, enabled us to perform optical recordings from different neurons simultaneously. We recently developed an SLM-two photon microscope (SLM-2PM) able to resolve the spatiotemporal organization of activity in acute cerebellar slices through simultaneous calcium signal acquisitions from multiple granule cells (Gandolfi et al., 2014). The SLM-2PM was optimized to investigate the effect of a mossy fibers high-frequency stimulation protocol (HFS), that is known to induce long-term potentiation and depression (LTP and LTD) in the cerebellum granular layer. HFS indeed caused long-term changes of granule cells calcium responses (Ca-LTP and Ca-LTD) with impressive variations in signal amplitude (Ca-LTP +351.81±19.14% n=25, Ca-LTD -62.86±1.77% n=19, 30 min after HFS). Loose cell-attached patch-clamp recordings were performed in order to elucidate the underlying mechanisms. The signal variation during Ca-LTP and Ca-LTD showed a similar dependency on the pre-stimulation state as observed with loose cell-attached and local field potential measurements (Mapelli and D'Angelo 2007, Sola et al. 2004). Interestingly, Ca-LTP amplitude changes were several times larger than those reported for synaptic currents measurements (D'Errico et al. 2009). The amplitude changes observed in Ca-LTP could be explained by a combination of changes in granule cells intrinsic excitability and synaptic transmission leading conjointly to a large increase in the probability of spike generation (Armano et al., 2000).

Disclosures: M. Tognolina: None. **L. Mapelli:** None. **E. D'Angelo:** None.

Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 622.06/SS18

Topic: E.02. Cerebellum

Title: What do the cerebellar parallel fibers do? A 40 year history of controversy and denial.

Authors: *J. M. BOWER;
Numedeon Inc., Ashland, OR

Abstract: Almost 40 years ago, as a first year graduate student newly joining the laboratory of Dr. Wally Welker at the University of Wisconsin, I had an idea for an experiment. Using high-density micro-electrode mapping techniques, Dr. Welker and his colleagues had recently discovered an unusual 'fractured' pattern of tactile afferent projections to the granule cell layers of the lateral hemispheres of the rat cerebellum. The maps were of a finer grain than any previously recorded and immediately suggested that overlying Purkinje cells would respond, via the parallel fibers, to tactile inputs originating from many locations on the body surface. Accordingly, I proposed that my thesis be based on constructing an equally fine-grained map of Purkinje cell activity in response to the multiple different types of tactile inputs projecting to the granule cell layer. In my thesis proposal I suggested that this experiment would be the first *in vivo* study using peripheral stimuli, to examine the expected parallel fiber beams of activated Purkinje cells and would therefore, allow direct study, for example, of the Marr/Albus theory of parallel fiber learning. Unfortunately, the Purkinje cell responses recorded in those experiments strongly and surprisingly suggested that parallel fibers did not directly drive Purkinje cell output and therefore, that theories like Marr/Albus theory that are based on strong parallel fiber inputs can not be correct.
This poster presentation will consider the ensuing almost 40 year effort to ignore, discount, obfuscate or dismiss this basic experimental result, as well as present the accumulated experimental data suggesting that the original result was in fact correct. The poster will also consider the implications of these results for theories of cerebellar function, like that of Marr/Albus, while also raising the epistemological question as to how neurobiologists can hope to make progress in understanding a structure as complex as the human brain, if they are unwilling, over 40 years, to question core assumptions about the structural organization of the circuits they study. The poster will also consider the benefits and risks of top down theories in directing experimental research as well as the potential importance and use of "bottom up" models in understand unexpected experimental results. Finally, the poster will describe a particular model-based hypothesis for what the parallel fibers may in fact be doing.

Disclosures: J.M. Bower: None.

Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 622.07/SS19

Topic: E.02. Cerebellum

Title: Diacylglycerol kinase gamma contributes cerebellar motor coordination through regulation of protein kinase C

Authors: R. TSUMAGARI[1], S. KIKUNAGA[1], Y. FUJIHARA[2], M. IKAWA[2], S. UEDA[1], M. YAMANOUE[1], S. KAKIZAWA[3], H. HARA[4], *Y. SHIRAI[1];
[1]Kobe Univ., Kobe, Japan; [2]Osaka Univ., Osaka, Japan; [3]Kyoto Univ., Kyoto, Japan; [4]Gifu Pharmaceut. Univ., Gifu, Japan

Abstract: Diacylglycerol kinase (DGK) is an enzyme that converts diacylglycerol to phosphatidic acid, and can attenuate PKC activity. So far, 10 subtypes of mammalian DGKs have been identified. Among them, DGKγ is abundantly expressed in neuron, especially in cerebellar Purkinje cells. PKCγ is also expressed there and involved in cerebellar motor coordination and the morphological control of Purkinje cells. PKCγ directly interacts with and phosphorylates DGKγ. Therefore, it is expected that DGKγ has important roles in cerebellar motor coordination and the morphology of Purkinje cells. However, the function of DGKγ in neuron is still unknown. So, we developed DGKγ knockout (KO) mice and tested their cerebellar motor coordination in rotarod test. DGKγ KO mice significantly fell in a short time compared to WT, suggesting the impairment of motor coordination. Next, we investigated the morphology of cerebellar Purkinje cells by Golgi staining to explore the mechanism underlying the impairment of motor coordination. The numbers of branches of cerebellar Purkinje cells decreased in the KO mice. In addition, the numbers of branches and length of dendrites in the primary cultured Purkinje cells from the KO mice significantly decreased, and the abnormality was rescued by PKC inhibitor, GF109203X, treatment. Furthermore, the phosphorylation of PKCγ was elevated in cerebellum from KO mice. These results suggested DGKγ contributes cerebellar motor coordination through regulation of PKC.

Disclosures: R. Tsumagari: None. **S. Kikunaga:** None. **Y. Fujihara:** None. **M. Ikawa:** None. **S. Ueda:** None. **M. Yamanoue:** None. **S. Kakizawa:** None. **H. Hara:** None. **Y. Shirai:** None.

Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 622.08/SS20

Topic: E.02. Cerebellum

Support: JSPS KAKENHI Grant-in-Aid for Young Scientists (B) (15K16086, KI)

JSPS KAKENHI Grant-in-Aid for Scientific Research B (24300115 and 16H02901, YH)

Title: Evaluation of roles of cerebellar Golgi and basket/stellate cells in the vestibuloocular reflex motor learning: a computational study

Authors: *K. INAGAKI, Y. HIRATA;
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Abstract: The vestibuloocular reflex (VOR) is one of the most popular model systems to study motor learning due to its clear function (stabilization of our vision) and ease in recording its input (head rotation) and output (eye movement) signals. The motor learning of the VOR requires the cerebellar flocculus. The flocculus receives sensory and motor information via mossy and climbing fibers, and outputs motor related activities to vestibular nuclei via Purkinje cell axons. Between these inputs and outputs lies a neuronal network containing rich inhibitory interneurons such as Golgi, basket, and stellate cells. While most of the previous studies on VOR motor learning have focused on responses of Purkinje cells, little attention has been paid to roles of cerebellar inhibitory interneurons due to a difficulty in identifying and recording those neurons in cerebellar cortex in behaving animals. Herein, we have constructed a computational model of the VOR that explicitly implements the anatomically realistic floccular neuronal network structure so that activities of each inhibitory interneuron can be evaluated. The model also allows us to knock-out any specific interneuron(s) at any timing of VOR motor learning. The model consists of 20 Purkinje, 10K granular, 900 Golgi, and 60 basket/stellate cells each of which is described as an integrate and fire spiking neuron model. These neuron models are connected, preserving convergence/divergence ratios between neuron types (Inagaki et al, 2011). As bases of VOR motor learning, climbing fiber spike timing dependent LTD and LTP have been implemented at parallel fiber - Purkinje cell synapses. We first confirmed that the model reproduces quantitatively eye velocities and Purkinje cell simple spike firing modulations during various visual-vestibular mismatch experiments in naïve monkeys, and simulated successfully changes in Purkinje cell activities along with VOR gains after prolonged exposure to VOR gain up and down paradigms. We then knocked-out Golgi or basket/stellate cells during various visual-vestibular mismatch experiments. As predicted, both granule and Purkinje cell DC firing rates increased after knocking-out Golgi cells, while only Purkinje cell DC firing rate increased

after basket/stellate cells knock-out. Interestingly, VOR gain before motor learning was affected neither by knocking-out Golgi cells nor basket/stellate cells. These results suggest that the interneurons play little role in maintenance of normal VOR, and its major role may be in acquisition of new VOR gains.

Disclosures: K. Inagaki: None. **Y. Hirata:** None.

Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

Location: Halls B-H

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Program#/Poster#: 622.09/SS21

Topic: E.02. Cerebellum

Support: grant from MEXT in Japan 25115716

Title: Training to induce adaptation of optokinetic response suppressed long-term depression in the cerebellar flocculus of mouse

Authors: *T. INOSHITA, T. HIRANO;
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Abstract: Long-term depression (LTD) at parallel fiber to Purkinje cell (PF-PC) synapses in the cerebellum has been regarded as a main mechanism for motor learning. Previous studies showed the necessity of LTD in motor learning by blocking pathways involved in LTD induction. However, some of reported motor learning failures might have been caused not by LTD deficiency but by other defects in the cerebellar function. In addition, normal motor learning under LTD suppressed conditions was reported. Thus, contribution of cerebellar LTD to motor learning has been debated. In order to address this issue in a different aspect, we have tried to clarify whether LTD occurred at PF-PC synapse during motor learning in the cerebellum. Adaptation of oculomotor reflex has been widely used as a model of cerebellum-dependent motor learning. Optokinetic response (OKR) is a type of oculomotor reflex, and works to stabilize an image on the retina during movement of a visual field. In order to induce OKR adaptation, we applied sinusoidal horizontal rotation of a screen with vertical stripes for an hour. During the training, eye movement got faster to catch up the screen movement better. Cerebellar flocculus is a regulation center of oculomotor reflex and H-zone in the flocculus regulates horizontal OKR, whereas V-zone regulates vertical OKR. After the OKR adaptation training of an adult mouse (from 8- to 10-weeks old), we prepared slices from the flocculus and performed whole-cell patch-clamp recording from Purkinje cells in H-zone of the flocculus. We failed to induce LTD in slices of flocculus prepared from OKR-trained mice, whereas LTD was

successfully induced in those from untrained mice. There was no significant difference in paired pulse ratio of EPSCs at PF-PC synapses, suggesting presynaptic release probability was not altered by the training. These results suggest that LTD occured at PF-PC synapses in H-zone of the cerebellar flocculus during OKR adaptation training.

Disclosures: T. Inoshita: None. **T. Hirano:** None.

Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

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Program#/Poster#: 622.10/SS22

Topic: E.02. Cerebellum

Support: MH 46904

MH 74006

Title: Using eyelid conditioning to study the learning of movement sequences by the cerebellum

Authors: *A. KHILKEVICH, L. NGUYEN, M.-M. RICHARDS, J. ZAMBRANO, M. D. MAUK;
Ctr. for Learning and Memory, Univ. of Texas At Austin, Austin, TX

Abstract: The cerebellum is necessary for precise performance of temporally complex motor tasks, such as sequences of movements. However, in common motor tasks several brain regions are involved, which makes it hard to isolate the specific cerebellar contribution. Eyelid conditioning is a well-establish cerebellar-dependent paradigm, where the movement (conditioned response, CR) is entirely driven by the cerebellum. Here we developed a new eyelid conditioning training protocol to study how sequences of movements are learned and generated by the cerebellum. During the surgery rabbits were implanted with stimulation electrodes in the middle cerebellar peduncle and mossy fiber stimulation was used as a conditioned stimulus (CS). Previous studies (Kalmbach et. al 2009) have established that if the temporal gap between the offset of mossy fiber stimulation CS and the onset of US is larger than 400 ms, animals are not able to learn using mossy fiber stimulation as the CS. Using this knowledge, experiments were designed to train animals to produce a series of CRs and to determine whether the expression of a CR can serve as the CS for a subsequent CR. For that, rabbits were first trained at ISI 500 ms with CS also lasting 500 ms. After acquiring stable CRs, animals presented training sessions with the same CS (500 ms), but with two potential times for US: US_1 at CS offset and US_2 at 600 ms from CS offset. On a given trial, if eyelid position value at time 499 ms from CS onset was lower

than the target (3mm, half-sized CR), US_1 was presented to maintain robust responding of the first CR. If it was higher than the target, US_1 was omitted and US_2 was presented. All rabbits successfully acquired dual-peak CRs with the timing of the second peak appropriate for US_2. That result suggests that the cerebellum uses the first CR as the "CS" for the next CR, as the mossy fiber stimulation CS is not able to support learning with a 600 ms gap between CS offset and US_2. Results from several control experiments further support this notion. Additionally, we have successfully used this paradigm to train animals to produce a left eye CR -> right eye CR sequence. For both paradigms, times of first and second CR onset and peak show very high ($r > 0.8$) trial-to-trial correlation, consistent with the first CR serving as the CS for the second CR. Recordings from Purkinje cells with US evoked complex spikes show that the first and second CRs and encoded in the same way by the cerebellar cortex. In sum, our results provide a clear demonstration that the cerebellum can use the information about the first part of the movement to learn the next one.

Disclosures: **A. Khilkevich:** None. **L. Nguyen:** None. **M. Richards:** None. **J. Zambrano:** None. **M.D. Mauk:** None.

Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

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Topic: E.02. Cerebellum

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Title: Climbing fibers reset Purkinje cell representations of behavior

Authors: M. L. STRENG, L. S. POPA, *T. J. EBNER;
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Abstract: The cerebellum is essential for online motor control. Requisite for elucidating the principles of cerebellar function is an understanding of the interaction between the two main excitatory inputs to Purkinje cells: climbing fibers and parallel fibers, which trigger and modulate complex spike (CS) and simple spike (SS) discharges, respectively. Although several

hypotheses have provided insights into the interaction between SS and CS discharges, including error encoding, altering responsiveness to parallel fiber inputs, and movement timing, none fully explain or are completely consistent with the spectrum of experimental observations. To address this, we evaluated the modulation of SS motor signals by climbing fiber input during a manual, pseudo-random tracking task. Accurate performance on this task requires continuously monitoring the constantly changing salient behavioral parameters and adjusting for mismatches in hand movement relative to target movement, and we have previously shown that SS firing contains predictive and feedback representations of these limb kinematics and performance errors during pseudo-random tracking.
The hypothesis that climbing fiber discharge alters signaling in the SS firing was tested by assessing the SS encoding strength for position (X and Y), velocity (VX and VY) and position error (XE and YE) before and after CSs using temporal linear regression analysis. Additionally, we assessed for changes in the mean behavior and SS firing rates pre and post-CS, as well as any evidence for CS rhythmicity. We find that climbing fiber discharge dynamically controls the information present in the SS firing, triggering robust and rapid increases and decreases in the SS encoding of motor signals in nearly all of the 40 Purkinje cells (> 90%). The increases and decreases in encoding are not due to differences in SS firing rates or behavior before or after a CS. Nor can the changes in encoding be attributed to any intrinsic CS rhythmicity. Our results suggest a novel hypothesis about the function of CSs, in which climbing fiber input resets the Purkinje cell from its current encoding state to one better matched to or dictated by the present conditions. The CS-coupled changes in SS encoding demonstrate that the representation of motor information is dynamic, focusing on the most task-relevant information and provide a novel neural correlate in support of the view that the motor system processes and uses different behavioral parameters to meet task requirements.

Disclosures: **M.L. Streng:** None. **L.S. Popa:** None. **T.J. Ebner:** None.

Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

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Topic: E.02. Cerebellum

Support: Erasmus University Fellowship

NWO ALW Veni

ERC Starter Grant

Title: Modulating modulation: Purkinje cell activity in impaired and enhanced compensatory eye movement adaptation

Authors: *M. SCHONEWILLE[1], H. ZHOU[1], T. KOUDSTAAL[2], C. I. DE ZEEUW[2];
[2]Neurosci., [1]Erasmus MC, Rotterdam, Netherlands

Abstract: Identifying the neural code underlying the process of learning in the brain is one of the main challenges in neuroscience today. In cerebellar motor learning several plasticity mechanisms have been identified to contribute to the adaptation of behavior, but the weight of each contributor, and how they are integrated in neural code remains unclear. Here, we recorded the activity of Purkinje cells, the output neuron of cerebellar cortex, prior to, during and directly following an eye movement training session. For optimal correlation, we compared the activity to that obtained under conditions of impaired and enhanced learning. We demonstrate a direct correlation between Purkinje cell activity and behavior, strengthened by the potential to rescue impaired learning with learning enhancers. Our results indicate that both rate and spatiotemporal coding contribute to cerebellum-dependent learning.

Disclosures: **M. Schonewille:** None. **H. Zhou:** None. **T. Koudstaal:** None. **C.I. De Zeeuw:** None.

Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

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Topic: E.02. Cerebellum

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Department of Biotechnology, Ministry of Science and Technology (DBT) BT/PR4983/MED/30/790/ 2012

Council of Scientific and Industrial Research (CSIR) 09/860(0142)/2012-EMR-I

Department of Atomic Energy, Government of India (DAE)

Title: Purkinje neurons in action: From single cells to ensembles

Authors: *M. SENGUPTA, V. THIRUMALAI;
Natl. Ctr. For Biol. Sci., Bangalore, India

Abstract: The cerebellum is critical for motor learning and is present in all vertebrates. Purkinje neurons (PNs) in the cerebellum are key contributors to the functions of the cerebellum and have been extensively studied. One prominent question has been whether these neurons exhibit bistability *in vivo*. Using the fact that the cerebellum is structurally conserved in vertebrates and that zebrafish larvae are highly amenable to *in vivo* electrophysiology, we performed targeted whole cell patch clamp recordings from un-anaesthetized larval zebrafish Purkinje Neurons and showed for the first time, that these neurons were spontaneously active and showed two modes of activity, tonic spiking and bursting. Moreover, the bursts during the bursting mode were driven preferentially by Climbing Fiber (CF) EPSPs. Finally, to further determine the functional implications of this activity, we recorded fictive motor signals extracellularly from the tail while simultaneously whole-cell patching Purkinje neurons. We showed that the bursting activity in these neurons correlated well with motor bouts but the latencies of the bursts to the onset of the motor bout varied within and across cells. Accordingly, we propose that these neurons encode a distributed representation of an efference copy.
To determine if these neurons indeed show a distributed code, we need to monitor populations of neurons at the same time by using calcium indicators. Zebrafish Purkinje neurons have been previously studied using calcium activity imaging in the context of the optomotor response (Ahrens *et al*.2012, Matsui *et al*.2014), however the physiological basis of this activity has not been determined. Our latest experiments therefore involve calcium imaging in single Purkinje neurons while simultaneously patching from them. Our data shows that the dendrites are more active than Purkinje somas. This activity correlates well to CF driven bursts and calcium spikes in these cells but not to tonic sodium spiking. Ensembles of Purkinje neurons recorded simultaneously with fictive motor bouts also show distributed activity.
Future experiments are aimed at studying the same in the context of motor learning.

Disclosures: **M. Sengupta:** None. **V. Thirumalai:** None.

Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

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Topic: E.02. Cerebellum

Support: NINDS Grant NS062771

NIH Grant AG008796

Title: Enhanced intrinsic excitability in cerebellar Purkinje cells following delay eyeblink conditioning in mice

Authors: *H. TITLEY[1], G. V. WATKINS[1], C. LIN[2], G. GRASSELLI[1], C. WEISS[2], J. F. DISTERHOFT[2], C. HANSEL[1];
[1]Dept. of Neurobio., Univ. of Chicago, Chicago, IL; [2]Dept. of Physiol., Northwestern Univ., Chicago, IL

Abstract: Cerebellar learning is classically assumed to depend on synaptic plasticity mechanisms, such as long-term depression (LTD) or potentiation (LTP), although it is now suspected that other mechanisms are involved, too. One such candidate mechanism is non-synaptic intrinsic plasticity in Purkinje cells. Intrinsic plasticity depends on a down-regulation of calcium-dependent SK-type K channels, and in the hippocampus it is known to play a role in the learning of trace eyeblink conditioning. However, it remains unknown to what extent Purkinje cell intrinsic plasticity contributes to the memory engram following a cerebellar learning task. Here we show that after delay eyeblink conditioning, Purkinje cells in lobule simplex / lobule HVI were more excitable than controls consistent with an increase of intrinsic excitability. Whole cell recordings were obtained from acute cerebellar slices from mice 48 hours after the final training session. Groups of mice received over a period of repeated training sessions either distinctly paired trials of a tone co-terminating with a periorbital shock (conditioned mice), unpaired trials of only a tone or a shock (pseudoconditioned mice) or neither a tone or a shock (naïve mice). We found that Purkinje cells from conditioned mice had a higher spontaneous firing rate than cells from controls, and were more excitable in response to gradually injected currents. Furthermore, following the stimulation of parallel fibers or climbing fibers, the Purkinje cell responses from conditioned mice were more excitable showing a greater depolarization, increased number of spikes and complex spike spikelets and a reduced afterhyperpolarizaton. This increase in excitability may suggest that SK-dependent intrinsic potentiation occurs during eyeblink conditioning, as the conditioned mice also show reduced excitability changes following the application of an intrinsic plasticity protocol, suggesting that this mechanism may be saturated in conditioned mice.

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Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

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Topic: E.02. Cerebellum

Support: JSPS KAKENHI Grant-in-Aid for Scientific Research B

Title: Role of the cerebellum in the initiation and termination of predictive optokinetic behavior in goldfish

Authors: S. MIKI[1], *R. BAKER[2], Y. HIRATA[1,3];
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Abstract: Optokinetic reflex (OKR) tracking is induced by the presentation of large field visual motion in nearly all vertebrate species. In response to visual velocity step stimuli the OKR exhibits two components: an initial rapid jump of eye velocity termed the "Direct" component, and a subsequent gradual rise termed the "Indirect" component (Cohen et al., 1977). Repetitive presentation of a visual velocity step stimulus induces OKR gain adaptation showing both an increase of the "Direct" component and a shorter time constant for the "Indirect" build-up (Marsh and Baker, 1997). When the repetitive visual velocity step stimulus is periodic with fixed ON/OFF periods, the OKR acquires a predictive behavior in which eye velocity starts to decrease before the termination of the stimulus ON period. This "Termination" component was first described in goldfish (Marsh & Baker, 1997), and later observed in carp and human (Miki et al., 2015). Herein, this predictive OKR behavior was further characterized by using periodic and non-periodic visual velocity step stimuli, and then the roles of the cerebellum were investigated by conducting acute and chronic cerebellectomy before and after the acquisition of the predictive behaviors. Goldfish were centered in a cylindrical water tank with eye coils binocularly placed on the cornea for eye position measurement by the search coil technique. In the periodic condition visual stimulation was projected onto tank wall and rotated clockwise at 20 deg/s for 8 seconds (ON period) and stopped for 8 seconds (OFF period). In the non-periodic condition, either the ON or OFF period was randomized between 1 and 15 seconds. After three hours of this visual paradigm in the periodic condition, eye velocity was observed to begin to increase significantly before initiation of the ON period. By contrast this "Initiation" component was not observed in the non-periodic condition. In chronically cerebellectomized goldfish using the periodic stimuli, there was no change in any of eye velocity parameters, i.e. the "Direct", "Indirect" "Termination" and "Initiation" components. In acutely cerebellectomized fish after periodic training, the adapted "Direct", "Initiation" and "Termination" components were lost, but some of the learned "Indirect" component remained. These results demonstrate that the cerebellum is required for both the acquisition and maintenance of the increased "Direct" component as well as the predictive "Initiation" and "Termination" of OKR tracking. Thus, the cerebellum appears necessary for acquiring the "Indirect" build-up, but maintenance of this component is not completely cerebellar dependent.

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Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

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Topic: E.02. Cerebellum

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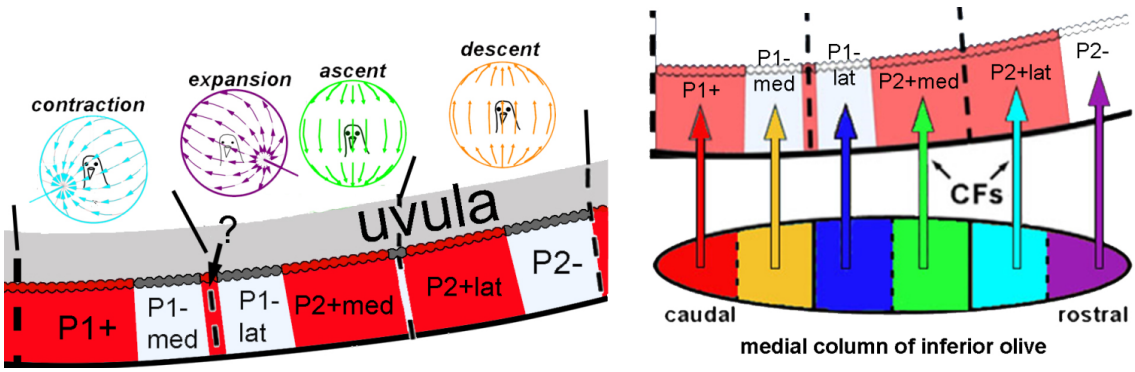
Title: Olivary Inputs to Zebrin stripes in the pigeon uvula: A retrograde study.

Authors: *D. WYLIE[1], I. CRACIUN[2];
[1]Dept. of Psychology, [2]Neurosciences and Mental Hlth. Inst., Univ. of Alberta, Edmonton, AB, Canada

Abstract: In the uvula of pigeons there are parasagittal zones defined with respect to the innervation of climbing fibers (CFs), the projection patterns of Purkinje cell (PC) outputs, and the response properties of PC complex spike activity (CSA) to "optic flow" stimuli. Additionally, the molecular marker zebrin II (ZII) is heterogeneously expressed in PCs such that there sagittal stripes of PCs with high ZII expression (ZII+), alternating with sagittal stripes of PCs with little to no ZII expression (ZII-). Previously we have shown that a ZII+/ - stripe pair in the uvula constitutes a functional unit, insofar as the CSA of all the ZII+ and ZII- PCs within the stripe pair prefer the same type of optic flow stimuli. As shown in the figure (left), there are three functional units in the uvula: (1) a medial ZII+/- pair where all PCs respond best to "contraction" optic flow; (2) an adjacent stripe pair where the PCs respond to either "expansion" or "ascent" optic flow patterns; and (3) a lateral stripe pair where all PCs respond best to "descent" optic flow. Although we have established that a ZII+/- stripe pair delineates a functional unit, other research, mainly in rodents, has emphasized ZII+ and ZII- bands receive input from different subnuclei of the inferior olive (IO). We carried out a retrograde tracing study to determine whether the ZII+ and ZII- bands of a stripe pair in the pigeon uvula receive differential input from the IO as opposed to input from the same area of the IO. Fluorescent Cholera toxin B (CTB) of different colours (red and green) was injected into ZII+ and ZII- bands of functional stripe pair, and the distribution of retrogradely labeled cells in the IO was analyzed. We found that injections in the ZII+ and ZII- bands of stripe pair did not result in retrograde labelling in different subnuclei, rather, spatially separate, but adjacent regions were labelled (see Figure right).



Disclosures: **D. Wylie:** None. **I. Craciun:** None.

Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

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Title: Functional differences in climbing fiber inputs to distinct cerebellar stripes during goal-directed behavior

Authors: ***S. TSUTSUMI**[1], N. HIDAKA[1], Y. ISOMURA[2], M. MATSUZAKI[3], K. SAKIMURA[4], M. KANO[1], K. KITAMURA[5];
[1]Dept. of Neurophysiol., Univ. of Tokyo, Tokyo, Japan; [2]Brain Sci. Inst., Tamagawa Univ., Machida, Japan; [3]Div. of Brain Circuits, Natl. Inst. for Basic Biol., Okazaki, Japan; [4]Div. of Cell.

Neurobio., Brain Res. Institute, Niigata Univ., Niigata, Japan; [5]Dept. of Physiol., Univ. of Yamanashi, Yamanashi, Japan

Abstract: Cerebellar climbing fiber (CF) inputs to Purkinje cells carry critical error signals and/or timing signals for motor control and learning. Recent studies suggest that Purkinje cells also play an important role in cognitive functions. CFs separately project from the higher order cortices and the periphery through the inferior olive to longitudinal clusters of Purkinje cells (cerebellar stripes), which are characterized by the presence or absence of aldolase C/zebrin II expression. However, detailed functions of these CF inputs to each cerebellar stripe during acquisition of skilled behaviors remain unknown. Here, we investigated the sensorimotor and cognitive aspects of CF inputs to the distinct cerebellar stripes in the cerebellar hemisphere (Crus II) by using chronic *in vivo* two-photon calcium imaging in mice learning a go/no-go auditory discrimination task. The lateral part of the Crus II received CF inputs that coincided with auditory cue; those to zebrin positive (Z+) stripes were initially synchronized to both go and no-go cues then concentrated to the go cue after cognitive learning, while those to zebrin negative (Z-) stripes were timed only to the no-go cue, which deteriorated throughout learning. CF inputs to the medial stripes were less timed and nonspecific to cues and zebrin expression. Population decoding of CF inputs clarified stripe-specific changes in the representation of lick rate, lick onset, cues and trials along with improvements in motor and cognitive behaviors. Our results indicate that CF inputs to the cerebellar stripes have distinct contribution in motor and cognitive processing. Such differential and parallel processing of sensorimotor and cognitive information in the distinct cerebellar microcircuits may collectively sophisticate goal-directed behavior.

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Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

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Topic: E.02. Cerebellum

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Bial Foundation

Title: Locomotor activity modulates delay eyeblink conditioning in mice

Authors: C. ALBERGARIA[1], T. N. SILVA[1], D. PRITCHETT[1], *M. R. CAREY[2];
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Abstract: In mice, locomotor activity modulates sensory responses across visual, auditory and somatosensory cortices. Here we show that locomotion also modulates behavioral performance in delay eyelid conditioning, a cerebellum-dependent form of classical conditioning. We monitored both eyelid closures and running speed in head-fixed mice running voluntarily on a running wheel. We found that increased locomotor activity was associated with earlier onset of learning and more frequent and larger amplitude conditioned responses. The correlation between running and learning was specific to conditioned (vs. unconditioned) responses. It held across animals, sessions, and trials, and for conditioned stimuli of various modalities. In contrast to the previously described modulation of sensory cortical processing, we found that the influence of locomotion on conditioned responses was dissociable from effects of arousal (as measured by changes in pupil size). Locomotor activity on a motorized treadmill also modulated learning in a speed-dependent manner, further suggesting that locomotor activity per se, rather than arousal, mediates the effect. To investigate the underlying neural circuit mechanisms, we used direct optogenetic stimulation of cerebellar mossy fibers as a conditioned stimulus. Conditioned responses elicited by optogenetic stimulation within the cerebellar cortex were also positively modulated by locomotor activity, indicating that enhanced upstream sensory processing cannot account for the modulation of the behavioral response. We conclude that locomotor activity modulates learned performance in delay eyelid conditioning through mechanisms that are distinct from those underlying modulation of sensory cortical responses and that act downstream of mossy fiber inputs to the cerebellar cortex.

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Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

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Program#/Poster#: 622.19/TT5

Topic: E.02. Cerebellum

Title: Corticotropin-releasing factor increases the excitability of deep cerebellar pojection neurons by modulating the HCN current

Authors: *A. M. LIBSTER, Y. YAROM;
Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: Corticotropin-releasing factor (CRF) is a neuropeptide that plays a major role in stress response. CRF is also released from climbing fibers that originate in the inferior olive nucleus and innervate the Deep Cerebellar Nuclei (DCN). Previous studies have shown that neurons of the DCN express CRF receptors and their firing rate is modulated by application of CRF. In the present study, we focused on the effect exerted by CRF on firing properties of the Big Projection Neurons (bPNs), and investigated the underlying biophysical mechanisms.
In vitro whole-cell patch recording were performed from bPN somata in coronal slices of cerebellum (prepared from C57BL/6 mice > p35). CRF (1-3 μM dissolved in HEPES buffered solution) was either added to the bath solution or injected locally via a patch pipette.
CRF application increased the firing frequency of the bPN. Local application of CRF to the bPN somata generated a prolonged episode of spikes firing, with a latency of several seconds, superimposed on a membrane depolarization. This response lasted for several minutes and was dependent on CRF concentration. Local application of CRF in the presence of TTX, revealed a small depolarization of the membrane potential, accompanied by a decrease in membrane resistance. voltage clamp recordings, with cell membrane potential, clamped to -70mV, have shown an increase in inward current, in the presence of CRF. Furthermore, this increase of inward current was blocked in the presence of ZD-7288, an Ih antagonist. The results suggest that the mechanism, underlying the CRF effect on bPN, is most likely an increase of Ih conductance.

Disclosures: **A.M. Libster:** None. **Y. Yarom:** None.

Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 622.20/TT6

Topic: E.02. Cerebellum

Title: Transcranial direct current stimulation delivered to the cerebellum affects the rate of fine motor learning

Authors: *J. K. SAMRA, R. E. SHIMIZU, A. D. WU, B. J. KNOWLTON;
UCLA, Los Angeles, CA

Abstract: The present study investigated the effects of cerebellar transcranial direct current stimulation (tDCS) on fine motor learning and subsequent performance on both practiced and novel motor sequence tasks. In the first study, participants practiced three distinct key press sequences presented in non-repeating, interleaved order while undergoing either anodal, cathodal, or sham stimulation. After the practice session, participants completed a transfer

session without receiving any stimulation; they practiced three novel sequences, also non-repeated and interleaved. Only those participants who received anodal stimulation during the practice phase showed evidence of sequence-specific learning (p = .033). Participants who underwent cathodal stimulation were significantly slower at performing the keypress task than participants in the anodal (p = .023) and sham (p = .024) groups. In the second study, a 24-hour delay was introduced between the practice and transfer sessions in an attempt to reduce potential fatigue caused by the practice session and stimulation. During this transfer session, participants who had received anodal tDCS during practice on the previous day showed significantly better transfer performance than those in the sham condition (p = .020). These results suggest that cerebellar tDCS can affect both the learning and performance of fine motor sequences. These results also suggest that anodal cerebellar tDCS during practice enhances the generalizability of the resulting motor programs.

Disclosures: J.K. Samra: None. **R.E. Shimizu:** None. **A.D. Wu:** None. **B.J. Knowlton:** None.

Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 622.21/TT7

Topic: E.02. Cerebellum

Title: Is mild essential tremor detrimental to activities of daily living?

Authors: *E. GOUBAULT[1], H. NGUYEN[2], S. BOGARD[3], F. AYACHI[3], G. FAUCHER[3], N. ROOFIGARI[3], É. LE[3], C. DUVAL[3];
[1]Sci. of Physical Activity, Univ. of Quebec At Montreal, Montreal, QC, Canada; [2]Univ. of Quebec at Montreal, Montreal, QC, Canada; [3]Univ. of Quebec at Montreal, Montréal, QC, Canada

Abstract: Background & aims. A recent trend in clinical research is the use of inertial sensor-based systems (ISS) to capture and assess voluntary and involuntary movements during activities of daily living (ADLs). Such technology may eventually prove useful to determine when involuntary movements become detrimental to daily activities. We recently proposed that the impact of involuntary movements on the voluntary motor behavior can be assessed using a Signal to Noise Ratio (SNR) approach, where the success of an activity depends on the magnitude of the intended voluntary movement (signal) and the magnitude of the involuntary movement (noise) present during that task. The aims of this study were to detect essential tremor (ET) during ADLs using ISS and determine whether they have a deleterious influence on the performance of those ADLs. **Method.** Healthy control participants (N=27, Age=63.93 ±7.67

years old) and participants diagnosed with ET (N=15, Age=62.4 ±8.25 years old) were asked to perform twice 11 ADLs presented randomly (reading, counting money, cutting-eating, eating soup, taking medicine, drinking water, picking-up an object, standing-sitting, walking, walking and taking care of a glass of water, walking and passing over an obstacle) while equipped with 17 inertial sensors (Animazoo IGS-180) positioned on each body segment. Participants diagnosed with ET, were either treated (N=5) or not (N=10) and possessed mild visible tremors at the time of the experiment. Using healthy controls data, we set success criteria based on the number of errors, as well as the time required to do the task. The threshold of success for time was set at the mean of healthy control participants plus 3 standard deviations. Power spectral analysis was used to assess tremor magnitude during each task. Chi-square tests were used for each task to determine the differences of failure in the two groups. **Results.** Amplitude of tremors during ADLs varied between 0.0021 g^2/Hz (correspond to a UPDRS score of 0.5) and 0.0485 g^2/Hz (correspond to a UPDRS score of 2) (mean=0.0158 g^2/Hz ±0.0146 g^2/Hz) for ET participants. Frequency of tremors during ADLs varied between 4.30 Hz and 6.44 Hz (mean=4.96 Hz ±0.64 Hz) for ET participants. Chi-square tests revealed no significant differences between the two groups in failure proportion ($p \geq 0.074$). **Conclusion.** The results of this study suggest that patients having mild treated or untreated ET were able to perform ADLs as well as healthy controls.

Disclosures: **E. Goubault:** None. **H. Nguyen:** None. **S. Bogard:** None. **F. Ayachi:** None. **G. Faucher:** None. **N. Roofigari:** None. **É. Le:** None. **C. Duval:** None.

Poster

622. Cerebellar Plasticity and the Role of Climbing Fibers

Location: Halls B-H

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Program#/Poster#: 622.22/TT8

Topic: E.02. Cerebellum

Support: NIH Grant R15NIMH106957

Title: Cerebellar neuromodulation and predictive processing in motor, cognitive, and social domains

Authors: ***A. M. D'MELLO**, C. I. C. THOMAS, C. J. STOODLEY;
American Univ., Washington, DC

Abstract: The cerebellum is thought to optimize both motor and non-motor behavior through the formation of internal models that enable effective predictive processing. Previous studies have shown that the posterolateral cerebellum is active during predictive processing in a variety of

tasks, yet the exact role of the cerebellum in such tasks has yet to be established. The right posterolateral cerebellum is thought to be involved in complex motor sequence learning, language, and social cognition. We investigated predictive processing in motor, cognitive and social domains following cerebellar neuromodulation with transcranial direct current stimulation (tDCS). Fifteen healthy young adults (15 male; mean age 19.9 ± 1.9 years) participated in three sessions (sham, anodal, and cathodal tDCS) each separated by one week. During each session, participants received 20min of 2mA sham, anodal, or cathodal tDCS over the right posterolateral cerebellum (1cm down and 4cm to the right of the inion). After neuromodulation, participants completed three task paradigms, each with both non-predictive and predictive components: a serial response time task (SRTT), a sentence completion task, and a social ball-playing task. Task and modulation conditions were counterbalanced within and between participants. We hypothesized that cerebellar tDCS would impact performance on trials requiring implicit predictive processing across paradigms: implicit sequence learning on the SRTT; semantic prediction on the sentence completion task; and implicit learning of reciprocal partnerships in the social ball-playing task. As anticipated, preliminary results suggest that cerebellar tDCS affected behavior when implicit learning and predictive processing were necessary, including motor learning (Friedman $p=0.012$), time to complete sentences with different levels of semantic predictability (Friedman $p<0.01$), and a trend effect on implicit learning of "good" vs. "bad" reciprocal partners in the ball-toss game (Friedman $p=0.08$). These findings support the idea that the cerebellum has a domain-general role in predictive processing, likely due to the implicit acquisition of information relevant to performance in both motor and non-motor contexts.

Disclosures: **A.M. D'Mello:** None. **C.I.C. Thomas:** None. **C.J. Stoodley:** None.

Poster

623. Human Finger Movements

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 623.01/TT9

Topic: E.04. Voluntary Movements

Title: Effects of training on independent finger movements

Authors: *S. A. WINGES, P. ACHARYA;
Kinesiology, Louisiana State Univ., Baton Rouge, LA

Abstract: Sense of hand shape and kinesthesia in our fingers relies on a variety of somatosensory information from skin and joint receptors of the hand. Dexterous fingers movements rely on this sensory information to aid everyday activities such as fastening buttons or snaps of a garment, picking up coins, or typing. It is understood that extensive practice is

required to become exceptionally skilled at dexterous finger movements such as typing or playing a musical instrument. The aim of this study was to examine whether enhanced resistance during tapping movements can be used to improve independent finger movements. If successful this could be a simple method to retrain these finger movements in individuals who have reduced dexterity. In healthy adults, individuated movements of middle, ring and little fingers are usually difficult to produce and simultaneously subjected to complementary or opposing movements of the adjacent digits. Thus we focused our study on training independent tapping movements of the middle, ring and little fingers in healthy individuals. Participants completed a handedness inventory and a questionnaire to assess what type of dexterous hand and finger movements they typically performed and if they had any particular hand and/or finger movement training. All participants were right-handed and performed tapping tasks with both their dominant and non-dominant hand. A flexible tape was used along the dorsal length of the finger during training. The task involved participants tapping one finger (instructed) for a period of twelve seconds. Individuated finger movements (Index, Middle, Ring, Little) were recorded using active markers (Codamotion 3D motion analysis system) placed on the distal end of each finger. Tapping movements during the central ten seconds of each trial were analyzed. Non-instructed finger movements were computed as the percentage of the displacement of the instructed finger movement. Non-instructed movement was largest for the Middle and Ring fingers prior to training and remained the largest after training as well. For some participants, by the end of training, the extent of non-instructed movement decreased below the pre-training levels although this effect was not sustained in the post test. It was concluded that this method may be effective but longer or multiple training sessions may be required to see sustained changes in non-instructed digit movement.

Disclosures: S.A. Winges: None. **P. Acharya:** None.

Poster

623. Human Finger Movements

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 623.02/TT10

Topic: E.04. Voluntary Movements

Support: EU FP7 project no 610967 (TACMAN)

VR 2011–3128

Title: Apparently purposeful variability in joint kinematics and fingertip forces during one- and two-digit manipulation

Authors: *B. B. EDIN;
Physiol. Section, Integrative Med. Biol., Umeå Univ., Umeå, Sweden

Abstract: Low variability is the hallmark of well-trained motor behaviors and this partly results from learning to cope with motor and sensory noise, i.e., that both the efferent output and the sensory signals that provides state and feedback information are inherently noisy. It has even been claimed that practically all human motor behaviors – from eye movements to hand trajectories – can be explained by optimal control models that minimize the impact of sensory and motor noise (Harris & Wolpert, 1998; Faisal et al 2008). There is, however, a kind of substantial variability at the actuation level that is unaccounted for by proposed models of motor learning (Van Beers 2009; Baddeley et al 2003; Diedrichsen 2005). For instance, Stimpel (1933) reported that when humans throw a ball towards a target, the dispersion of the elbow angle and release velocity are substantially larger than that of the final position hit by the ball. In the same vein, Bernstein (1967) described the highly variable trajectory of hammering movements by a blacksmith despite skillful target hitting.
We let participants pull and push an object using either the index finger or both the index and middle finger while recording the object's position and velocity, the fingertip forces, and the finger joint angles. No constraints were imposed on the kinematic or kinetics of the task but the timing of individual pull-push movements was provided by auditory cues. Series of pull-and-push movements were performed with different surface friction while the servo-controlled object emulated either viscosity or inertia.
The participants solved the task in highly idiosyncratic manners, e.g., increased the surface normal force or decreased the applied surface tangential force with decreasing surface friction. In contrast, the intrasubject variability was low in terms of the "objective task". Importantly, despite this low task variability, all participants showed a marked variability from trial-to-trial with respect to both joint kinematics and how the surface tangential force was partitioned between the digits. Moreover, the kinematics and kinetics did not covary.
Three possible reasons for this variability are proposed: (1) to counteract fatigue; (2) to keep sensorimotor transformations updated; and (3) to allow explorations of behaviors that may be useful under kinematic restrictions or when managing perturbations.

Disclosures: B.B. Edin: None.

Poster

623. Human Finger Movements

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 623.03/TT11

Topic: E.04. Voluntary Movements

Support: CIHR Grant (125 915)

Title: Factors affecting cognitive-motor integration impairment and recovery in children post-concussion

Authors: *M. DALECKI[1], D. GORBET[2], A. MACPHERSON[1], L. E. SERGIO[2];
[1]Sch. of Kinesiology and Hlth. Sci., [2]Sch. of Kinesiology and Hlth. Science, Ctr. for Vision Res., York Univ., Toronto, ON, Canada

Abstract: Cognitive-motor integration (CMI) is required to perform rule-based visuomotor transformations, for example, when the spatial locations of the guiding sensory information and required motor action differ. We previously observed prolonged CMI deficits in children with a history of concussion who were classified as asymptomatic by current return to play protocols (Dalecki et al. 2016). In the current study, we examine factors that may influence the decline and recovery of CMI performance in children with a history of concussion.

Sixty-four children with a history of concussion (mean 14 months post-concussion; mean age 13 yr) and sixty-two age-matched controls with no previous concussion performed two eye-hand coordination tasks. In the direct interaction task, participants slid their finger on a touch screen to move a cursor from a central target to one of four peripheral targets. In the CMI task, targets were in a different plane from hand motion, and feedback was 180° reversed (i.e. decoupling between vision and action). We analyzed movement timing and trajectory variables, as well as the effects of age, sex, and sports experience (years of play).

In agreement with our previous study, we observed significant CMI movement timing and execution deficits in children with a history of concussion, and a significant relationship between the time since last concussion and CMI performance. In addition, in children with a concussion history, we found a significant relationship between CMI performance and sport experience, such that children with concussion history and higher sport experience outperformed those with concussion history and lower sport experience. Both effects were not present in the control group and could not be attributed to a general effect of age or sex. Furthermore, children with concussion history and higher sport experience had quicker CMI recovery times compared to their lower sport experience peers.

These results indicate an important role of sport experience in recovery from concussion, and are in line with other studies from our lab showing CMI deficits in young varsity athletes but fewer deficits in young elite athletes with a concussion history (Brown et al. 2015; Hurtubise et al. 2015). We suggest that children and young adults with a concussion history but higher sport experience have more motor skill-related neurological reserves. This reserve may in turn reduce CMI performance declines and improve CMI recovery post-concussion. Imaging studies suggest the neural correlates of these reserves exist in more efficient fronto-parietal networks, which are known to heavily contribute to CMI.

Disclosures: **M. Dalecki:** None. **D. Gorbet:** None. **A. Macpherson:** None. **L.E. Sergio:** None.

Poster

623. Human Finger Movements

Location: Halls B-H

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Program#/Poster#: 623.04/TT12

Topic: E.04. Voluntary Movements

Support: Internal grant by the authors' institution

Title: Perception and motor control rely on different spatial reference frames.

Authors: *O. L. BOCK, N. A. BURY;
German Sport Univ., Koln, Germany

Abstract: We have reported before that in absence of visual cues, judgements of the vertical are aligned mostly with gravity, less often with the long axis of the own body, and rarely otherwise (Bury and Bock, SfN 2015, 335.17). Here we evaluate whether the same alignment holds for vertically oriented hand movements. Twenty-four young, healthy volunteers were blindfolded, tilted into different angles of roll, and asked to silence an alarm by flipping a switch “down” with their dominant hand. The switch was constructed such that it could be flipped in any direction in the participants’ frontal plane. Before the actual experiment, all volunteers practiced the task while standing upright with eyes open (i.e., with congruent visual, gravitational and egocentric information about “down”). We found that 20/24 participants deflected the switch in accordance with their body orientation, rather than in accordance with gravity. Some of these participants deflected the switch consistently towards their own feet, while others deflected it consistently in other egocentric directions. Among the remaining four participants, two deflected the switch in accordance with gravity while the other two couldn’t be classified unequivocally. We conclude that motor control may rely on distinct internal representations of the vertical, and that the predominance of egocentric responses may adversely affect human operation under water and in spaceflight.

Disclosures: O.L. Bock: None. **N.A. Bury:** None.

Poster

623. Human Finger Movements

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 623.05/TT13

Topic: E.04. Voluntary Movements

Support: Indonesian Endowment Fund for Education

Title: Extrinsic and intrinsic dynamics in visuomotor tracking

Authors: *D. SUSILARADEYA, F. GALÁN, K. ALTER, A. JACKSON;
Inst. of Neurosci., Newcastle Univ., Newcastle Upon Tyne, United Kingdom

Abstract: During visuomotor tracking of slow targets, humans produce submovements at frequencies around 2 Hz. This rhythmicity could reflect properties of the extrinsic feedback loop, with submovement frequency determined by visual processing delays. Alternatively, an intrinsic rhythmicity within the motor system could enhance motor responses at submovement frequencies. We tested these two hypotheses using 2D bimanual finger force tracking of a circularly-moving target. We added two experimental manipulations of visual feedback: time delays (of 100-400 ms) and sinusoidal perturbations (at 1-5 Hz).
Perturbations produced time-delayed force responses which at submovement frequencies were phase-shifted by 180° leading to large cursor errors. Moreover, the frequency of both submovements and perturbations associated with large errors decreased with additional feedback delay. These observations are consistent with time delays in the extrinsic feedback loop driving submovements. However the frequency distribution of submovements, and the force response to perturbations at different frequencies, suggested that these time delays increased slightly with frequency. Moreover, the force response to perturbations at 2-3 Hz had a larger amplitude that that for higher or lower frequencies, and this was independent of delay time. These results are consistent with intrinsic dynamics within the feedback loop causing a frequency-dependent phase shift and a resonance at submovement frequencies.
We modelled a feedback controller driven by discrepancies between delayed sensory predictions (based on efference copy) and delayed feedback of errors. Intrinsic dynamics within the feedback loop were represented by a first order recursive filter. This model was able to reproduce all the features of our data. Stable tracking required that sensory prediction delays accurately matched actual feedback delays, suggesting that humans can quickly adjust this in the presence of artificially increased delay. However, the intrinsic dynamics of the feedback loop remained constant across conditions, suggesting that these are instantiated by static or slowly adapting circuits. We speculate that these intrinsic dynamics could reflect the recursive properties of a state estimator that combines an internal prediction with noisy, delayed sensory information.
In conclusion, the frequency composition of human visuomotor tracking in the presence of

perturbed visual feedback reveals the contribution of both extrinsic and intrinsic dynamics to the generation of submovements.

Disclosures: **D. Susilaradeya:** None. **F. Galán:** None. **K. Alter:** None. **A. Jackson:** None.

Poster

623. Human Finger Movements

Location: Halls B-H

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Program#/Poster#: 623.06/TT14

Topic: E.04. Voluntary Movements

Support: Cognitive science research initiative (CSRI),DST, India

Title: Hand postures can be classified and reconstructed from kinematic synergies identified through linear approaches

Authors: ***N. S. BHATT**, V. SKM;
Applied Mechanics, Indian Inst. of Technol. Madras, Chennai, India

Abstract: Human hand is a sophisticated, complex and redundant mechanical system controlled by a relatively well developed central nervous system. Redundancy in the hand helps humans to perform tasks such as making postures or grasping objects with ease, especially in uncertain environments. In our study, seven subjects (3 females) performed 70 different postures including daily life postures, ASL (American Sign Language) and aesthetic postures (Indian dance form Bharatnatyam). Each posture was 5 trials each (a total of 350 trials). Sixteen (15+1 reference) 1.8 mm Liberty Electromagnetic tracking sensors (Polhemus, USA) were placed on each finger phalanx and we measured position and orientation data at 120Hz. We considered the hand as a 21-DOF system and computed 21 joints (4 DoFs for the digits and 5 DoF for the thumb). Our results suggest that there is a linear relationship between DIP-PIP joint for all fingers across subjects. For projecting data into linear space we employed two techniques, Principal component analysis (PCA) and Unsupervised Linear Discriminant Analysis (ULDA). We found PCA with 2 PCs gives reconstruction error of ±1 degree, which shows PCA with first two PCs effectively reduce dimensionality. Our results further show that adding 3rd PC reduces the error to ±0.5 degree but describes the finer features of the postures. In some applications, dimensionality reduction alone may not be sufficient. To discriminate two postures from each other we employed the K-Means supervised learning classifier on the dimensionality reduced data. Our results from this analysis suggest that some aesthetic postures and ASL are misclassified, implying that there is a measure of similarity between these postures. We suggest that these two linear dimensionality reduction techniques (PCA & LDA) come with a trade-off between

accurate joint angle reconstruction and posture classification. We further suggest that these two techniques must be used to complement information from each other, but not as a replacement for the other. We further suggest that analyzing such high dimensionality data within the framework of non-linear approaches such as Gaussian Process Latent Variable method (GPLVM) may offer more insights into the true nature of control for this highly redundant system.

Disclosures: **N.S. Bhatt:** None. **V. Skm:** None.

Poster

623. Human Finger Movements

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 623.07/UU1

Topic: E.04. Voluntary Movements

Support: CIHR

NSERC

Title: Human dorsal premotor cortex transfers information to primary motor cortex hand representation during the preparation for an upcoming grasp: a dual-site TMS study

Authors: ***M. VESIA**[1,2], J. C. CULHAM[3], G. JEGATHEESWARAN[1,2], R. ISAYAMA[1,2], A. LE[4], R. CHEN[1,2];
[1]Univ. of Toronto, [2]Krembil Res. Inst., Toronto, ON, Canada; [3]Dept. of Psychology, The Brain and Mind Inst., Western Univ., London, ON, Canada; [4]Ctr. for Vision Res., York Univ., Toronto, ON, Canada

Abstract: Dorsal premotor cortex (PMd) is involved in the selection, preparation and execution of voluntary action. Monkey neurophysiology and human neuroimaging studies indicate that PMd encodes grasp. We hypothesized that the functional connection between PMd and the primary motor cortex (M1) is increased during preparation for grasping. Paired-pulse transcranial magnetic stimulation (TMS) with two coils was used to test functional interactions between left PMd and ipsilateral M1 (and within left M1) while at rest or during preparation to grasp objects with either a precision grip or a whole-hand grasp. The test stimulus (TS) was applied to M1 with a small branding iron style coil (50 mm diameter). The TS intensity was adjusted to evoke motor evoked potential (MEP) amplitudes of ~1mV. Another branding iron coil (40 mm diameter) was used to deliver the conditioning stimulus (CS) to PMd. The CS was set at 90% of the active motor threshold (AMT). Interstimulus intervals (ISI) of 4, 6, and 8 ms between CS and

TS were used. We show that when planning to grasp objects with either a precision grip or a whole-hand grasp, PMd facilitates M1 excitability in the hand representations. We also found that MEP amplitudes in hand muscles during grasp preparation are associated with the pattern of muscle activity used in the upcoming grasp. The degree of MEP facilitation was larger when conditioning PMd compared to conditioning M1 (paired-pulse M1). Consistent with findings reported in the monkey, these results provide causal evidence that human PMd transfers grasp-related information to M1 hand representation during the preparation for an upcoming grasp. This new basic understanding of the high-level, intention-related activity in human frontal circuits during goal-directed hand actions may better inform decoding algorithms used to operate neural cognitive prosthetic devices.

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Poster

623. Human Finger Movements

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 623.08/UU2

Topic: E.04. Voluntary Movements

Support: Medical Research Council/AstraZeneca Mechanisms of Disease Grant

Title: Effects of a GABA(A)alpha2,3 modulator AZD7325 on corticospinal excitability in healthy volunteers

Authors: ***G. SAMUSYTE**[1], L. WEBBER[2], J. ROTHWELL[1], M. KOLTZENBURG[1]; [1]Sobell Dept. of Motor Neurosci. and Movement Disorders, UCL Inst. of Neurol., London, United Kingdom; [2]AstraZeneca R&D, Alderley Park, United Kingdom

Abstract: Paired-pulse transcranial magnetic stimulation (TMS) is a non-invasive method to study corticospinal excitability (CSE) and the action of GABA modulating drugs in humans. Short interval intracortical inhibition (SICI), one of the biomarkers of CSE, was shown to be enhanced by a single dose of benzodiazepines, but not drugs acting selectively through GABA(A)alpha1 receptors. As alpha5 subunits of GABA(A) receptors are less densely expressed in the cortex than alpha2,3 subunits, it has been suggested that GABA(A)alpha2,3 receptor signalling is responsible for the modulatory effects on SICI. Availability of the novel GABA(A)alpha2,3 positive allosteric modulator AZD7325 allowed us for the first time to investigate this hypothesis in humans. GABA(A)alpha2,3 modulation of CSE was assessed in 12 healthy male volunteers (24±4 years) in a phase I randomized, double-blind, placebo-controlled,

3-way cross-over study. SICI was obtained at baseline and 1, 2, and 8 hours after a single dose of 2 mg, 10 mg of AZD7325 or matched placebo. To avoid “floor effect”, a range of conditioning stimuli (CS) was used (50-80% of resting motor threshold (RMT), interstimulus interval 2.5 ms). Test stimulus (TS) intensity was set to produce a response of 1 mV. Absolute and relative SICI change from baseline was assessed. Increase in inhibition was expected at the time of maximum plasma concentration (Cmax) of the drug, i.e. 1-2 hours post-dose. In addition, sedation and psychomotor performance were assessed. Baseline CSE parameters did not differ between the treatments. AZD7325 did not have an effect on RMT, but both 2 and 10 mg doses increased TS intensity at Cmax when compared to placebo. There was a consistent, however non-significant, absolute and relative increase in SICI at CS 60% and 70% RMT at Cmax after the intake of 10 mg of AZD7325 (absolute change of 6-17% of test response, relative change of 7.5-11%). Interestingly, the largest increase in SICI at CS 70% RMT (relative change of 26%) was observed at 8 hours after intake of AZD7325, and was close to reaching statistical significance after 10 mg dose. There was no significant effect of AZD7325 on sedation and psychomotor performance when compared to placebo. In conclusion, 2 and 10 mg doses of AZD7325 were non-sedating and did not impair psychomotor performance. At Cmax, increases in SICI with AZD7325 did not reach statistical significance, but increased TS intensity is suggestive of a possible rightward shift and/or change in slope of the input-output curve. Larger studies employing higher doses of AZD7325 and an active comparator are needed to further explore the hypothesis of GABA(A)alpha2,3 modulation of SICI.

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Poster

623. Human Finger Movements

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 623.09/UU3

Topic: E.04. Voluntary Movements

Title: Human somatotopic organization during finger movement revealed by gaussian population receptive fields

Authors: *W. SCHELLEKENS[1,2], N. F. RAMSEY[1,2];
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Abstract: Cortical sensorimotor areas in the human brain have been shown to exhibit somatotopic organizations at least at the macroscopic level [1]. However, the existence of a somatotopic organization on a microscopic level (e.g. individual digits) is much less certain, especially in motor areas [2]. The population Receptive Field (pRF) approach, that has been proven successful in retinotopic mappings of visual cortex [3], could shed more light on this issue. The current study investigates if an orderly somatotopy of finger representations can be obtained using fMRI and pRF analysis.
During a 7T fMRI session, 6 healthy volunteers executed a finger movement task that required the sequential flexion or extension of all fingers of both hands separately. During the task the subject's finger positions transitioned from flexed towards extended or vice versa. Similar to pRF analyses in visual neuroscience, a Gaussian convolved with a hemodynamic response function was fitted to the timeseries of each voxel, thereby obtaining the center and sigma of a voxel's best Gaussian fit. The Gaussian center illustrates a voxel's preferred finger representation, while the sigma illustrates the degree of response to adjacent fingers. The presence of an orderly somatotopy of individual finger representations was assessed for several Brodmann areas using a linear regression on the coordinates of the obtained centers.
Somatotopic organizations of finger representations were found in all subjects in the contralateral hemisphere with respect to the cued hand. During flexion, the following Brodmann areas showed a significant somatotopy in somatosensory cortex: BA1 ($T_{(5)}$=9.99, p<.001); BA2 ($T_{(5)}$=2.77, p=.039); BA3a ($T_{(5)}$=5.68, p=.002); BA3b ($T_{(5)}$=5.18, p=.004), and motor cortex: BA4p ($T_{(5)}$=5.44, p=.003); BA6($T_{(5)}$=3.64, p=.015). During extension only somatosensory cortex showed somatotopic organizations: BA1 ($T_{(5)}$=8.48, p<.001); BA3a ($T_{(5)}$=3.77, p=.013); BA3b ($T_{(5)}$=4.61, p=.006).
In the current study we show that the somatotopic organization of finger representations can be obtained in individual subjects' sensorimotor cortex with the use of a Gaussian pRF approach. The pRF analysis results in a preferred finger representation per voxel, as well as a degree of response to finger representations adjacent to the preferred one. The pRF approach shows that

motor cortex has a detailed microscopic somatotopic organization, and that its classification can be revealed with integrative models of sensorimotor activation.
1 Penfield W, Boldrey E (1937) Brain 60:389–443.
2 Sanes JN, Schieber MH (2001) Neuroimage 13:968–974.
3 Dumoulin SO, Wandell BA (2008) Neuroimage 39:647–660

Disclosures: W. Schellekens: None. **N.F. Ramsey:** None.

Poster

623. Human Finger Movements

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 623.10/UU4

Topic: E.04. Voluntary Movements

Title: Effects of absolute force level and direction of changes in force on accuracy in a cyclic isometric low level force pinch task

Authors: M. LEWIS, K. MULLER, M. DUNN, R. T. EAKIN, *L. D. ABRAHAM; Kinesiology and Hlth. Educ., Univ. of Texas at Austin, Austin, TX

Abstract: Previous research has described relationships between force production accuracy and absolute force level in static and dynamic force production tasks. This study examined whether greater error in a cyclic visuomotor isometric force tracking task would occur at the lower force reversal point than when the same force level was the high end of a similar task performed over a lower force range. Thirty right-handed volunteer participants, 6 men and 24 women (18 - 30 yrs), free from any neurological disorder or physical ailment, were tested. The participants performed an isometric pinch tracking task, which required generating matching forces with both the thumb and index finger of the preferred hand to move a cursor on a computer screen up and down a 45-degree diagonal line, trying to stay coincident with a track ball cycling between two force levels four times for each trial. The forces required to complete the task were scaled so that the peak force required for each digit was 12% of that individual's mean maximum voluntary contraction (MVC) digit flexion force. All participants were tested in ten trials under each of two conditions in random order. One condition required cycling between force levels of 3% MVC and 6% MVC; the other condition required cycling between 6% and 12% MVC. Tracking error data were analyzed from the ten performance trials by each participant at the 6% MVC level; in one condition this level was at the high end of the task range and in the other condition this level was at the low end of the task range. The four cycles of the track ball in each trial yielded three reversal data points at the 6% MVC force level. Consistent with the force / force variability principle, the results showed that for both the thumb and index finger absolute error was greater

at higher force levels than lower force levels, while in each range greater error was seen at the low end reversal than the high end reversal. Importantly, as previously reported by Park (2012), higher error was seen for both digits at the 6% MVC level when that was the low end of the target force range than when that was the high end of the force range. This result supports the idea that the task of precisely reversing a decreasing force level to begin increasing pinch force is more difficult than the task of reversing an increasing force level to begin decreasing pinch force. Underlying mechanisms may be related to the dynamics of motor unit recruitment and isometric muscle force production during force reversals at low force levels.

Disclosures: **M. Lewis:** None. **K. Muller:** None. **M. Dunn:** None. **R.T. Eakin:** None. **L.D. Abraham:** None.

Poster

623. Human Finger Movements

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

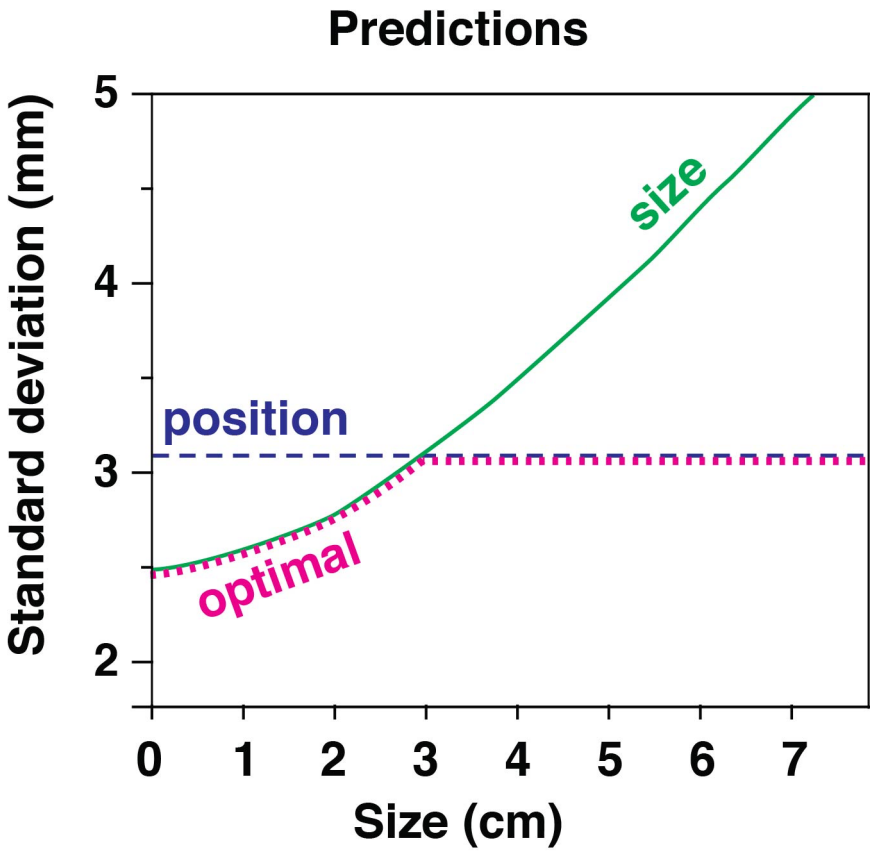
Program#/Poster#: 623.11/UU5

Topic: E.04. Voluntary Movements

Title: The lack of effect of a visual size illusion on grip aperture is independent of object size.

Authors: *J. B. SMEETS, E. BRENNER;
Vrije Univ. Amsterdam, Amsterdam, Netherlands

Abstract: There is an extensive literature debating whether visual size illusions influence the peak grip aperture in grasping. We found no effect for 7cm diameter disks on a Ponzo illusion (Brenner & Smeets, EBR, 1996). We interpreted this as evidence that grasping is not based on a visual estimate of size. Most studies that did find an influence of illusions used approximately 3cm diameter disks embedded in an Ebbinghaus illusion. Could it be that people do use size information for smaller objects because they combine information to maximise precision, and for smaller objects size judgments are no longer less precise than judgments of position (Smeets & Brenner, Current Biology, 2008). In order to avoid any possibility of parts of the illusion being interpreted as obstacles, we tested this possibility using a modified diagonal illusion. Participants grasped both small (1.5-2.5 cm) and slightly larger (4-5 cm) objects. The illusion had an effect of more than 10% on perceptual judgements, irrespective of object size. For the peak aperture during grasping movements, the effect of the illusion was negligible (less than 0.5%), again independent of object size. We conclude that the reported disagreement on the effect of illusion is not due to using differently sized objects.



Disclosures: **J.B. Smeets:** None. **E. Brenner:** None.

Poster

623. Human Finger Movements

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 623.12/UU6

Topic: E.04. Voluntary Movements

Title: Measurements of recovery from finger muscle fatigue

Authors: ***M. O. CONRAD**, S. JAMES;
Sch. of Engin. and Computer Sci., Oakland Univ., Rochester, MI

Abstract: Localized muscle fatigue results from several factors including both metabolic and structural changes occurring with prolonged or repetitive muscle use. Methods of evaluating muscle fatigue include both techniques that track maximum force capacity of a muscle as well as

the analysis of surface electromyography (EMG) under controlled conditions to evaluate the amplitude and frequency of the EMG signal. While prior studies have extensively used such measures to evaluate the onset of fatigue, relatively few studies have focused on quantifying fatigue throughout the recovery phase. The aim of this study is to evaluate various methods of analyzing force and EMG signals to identify the most accurate method for analyzing muscle recovery post-fatiguing contractions. Muscle fatigue was evaluated in the first dorsal interosseous (FDI) and thenar eminence of the thumb during voluntary muscle contractions using a Biometrics DataLOG System (Biometrics Ltd, UK). Each subject exerted lateral pinch force on a custom pinch dynamometer completing two different fatiguing protocols. For the continuous protocol subjects exerted 50% MVC until exhaustion. During the intermittent protocol the subjects alternated 5 s exertions (50% MVC) with 5 s rest periods for 6 min. After every minute a MVC was recorded to note any changes in normalized force production. For one hour after completion of the fatiguing contraction muscle activity was monitored every 10 min. Normalized values of RMS amplitude, median frequency, and peak force exertions were compared between protocols and throughout the recovery phase of the experiment. Results indicate for both muscles during the intermittent protocol median frequency analysis indicated greater % fatigue than that observed in the RMS amplitude. The median frequency analysis appears more sensitive to recovery of muscle post-fatiguing contractions however median frequency and RMS results appear to be correlated for each muscle. Further research must be conducted to further understand the exact mechanisms contributing this these observations.

Disclosures: M.O. Conrad: None. **S. James:** None.

Poster

623. Human Finger Movements

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Program#/Poster#: 623.13/UU7

Topic: E.04. Voluntary Movements

Support: BBSRC (UK)

FWO Odysseus (Belgium)

Title: Visuo-haptic asynchrony alters object lifting dynamics and weight perception

Authors: V. VAN POLANEN[1], *M. DAVARE[2,1];
[1]Movement Control and Neuroplasticity Res. Group, KU Leuven, Leuven, Belgium; [2]Inst. of Neurol., London, United Kingdom

Abstract: Object lifting requires precise scaling of fingertip forces according to the weight of the object. When lifting an object from a surface, lift-off occurs when the applied vertical load force overcomes the object weight. Thus a weight percept can be formed early during the loading phase of a lifting motion. Different sensory events inform the brain about the time of object lift-off: visual cues and haptic inputs (proprioception and fingertip mechanoreceptors) indicate when the object releases contact with the surface and starts to move. In real life conditions visual and haptic inputs about lift-off occur simultaneously. Here we used a virtual reality environment where a delay between visual and haptic feedback could be introduced in order to determine the relative contribution of vision and haptics in controlling fingertip force scaling and object weight perception. Participants were required to lift virtual objects simulated by a 3D computer screen with their thumb and index finger attached to force-feedback robots (Phantom). In half of the trials, vision was delayed by 100 or 200 ms, resulting in an asynchrony between haptic and visual information. Participants lifted two objects sequentially and one of the two lifts had a visual delay. They were subsequently asked to report which object was heavier. Object mass was varied according to an adaptive staircase procedure to determine the mass at which an object lifted with delay felt equal to an object lifted without delay. We found that objects are perceived as being heavier when vision is delayed with respect to haptic feedback. In addition, force scaling is altered in response to the altered visual information. This indicates that visual information about lift-off plays a significant role in controlling force scaling and forming weight perception. Because a visuo-haptic asynchrony mainly alters perception of sensory events occurring during lift-off and to a lesser extent during the static holding phase, our results suggest an important role of the dynamic changes occurring during object loading in generating weight perception.

Disclosures: V. van Polanen: None. **M. Davare:** None.

Poster

623. Human Finger Movements

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 623.14/UU8

Topic: E.04. Voluntary Movements

Title: The effect of using a stylus in different planes and feedback configurations

Authors: J. M. ABDALLA[1], S. C. MONTGOMERY[1], C. A. AIKEN[2], *A. W. VAN GEMMERT[1];
[1]Kinesiology, Louisiana State Univ., Baton Rouge, LA; [2]Integrative Physiol. and Hlth. Sci., Alma Col., Alma, MI

Abstract: Since the 1970s, pen and digitizer-tablets were used as input devices with computers to investigate handwriting and drawing performance. This configuration's drawback is that the feedback and movements are not displayed in the same plane. Recent technological advances allow the display to be integrated within the digitizer (i.e., display-tablets), thus allowing feedback to be displayed in the same plane as the movement. Furthermore, whereas the traditional setup resulted in feedback displayed in the vertical plane on a monitor, display-tablets allow us to display feedback in the vertical and horizontal planes. As a result the question emerges whether vertical and/or horizontal movements with feedback in the same plane do differ from each other and whether movements are affected if the feedback is shown in the vertical plane while the movement occurs in the horizontal plane (i.e., the traditional configuration). The study's purpose was to identify any possible effects on motor performance while executing different graphic tasks in the three different configurations. Participants were asked to perform a handwriting-like and graphical aiming task. Preliminary data showed that the plane's orientation of the executed movement does not affect performance significantly. However, when the executed movements are dissociated from the feedback (i.e., the feedback is not in the same plane as the movements) performance deteriorates significantly (i.e., longer durations; less smooth movements). These results suggest that general conclusions drawn upon findings of research studies using protocols in which the feedback was displayed on a monitor in the vertical plane while the movements were made on an opaque digitizer tablet placed on a table should be accepted with caution.

Disclosures: J.M. Abdalla: None. **S.C. Montgomery:** None. **C.A. Aiken:** None. **A.W. Van Gemmert:** None.

Poster

623. Human Finger Movements

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Program#/Poster#: 623.15/UU9

Topic: E.04. Voluntary Movements

Support: NSERC Grant 6313-2012

NSERC CREATE Grant 371161-2009

Title: size constancy in grasping

Authors: *J. CHEN[1], R. RINAS[2], I. SPERANDIO[3], M. A. GOODALE[1];
[1]The Brain and Mind Inst., [2]Dept. of Psychology, Univ. of Western Ontario, London, ON, Canada; [3]Sch. of Psychology, Univ. of East Anglia, Norwich, United Kingdom

Abstract: When we look at an object from different distances, the size of the image of the object projected onto our retina varies, yet we still perceive the object to be the same size. This phenomenon, called size constancy, is not only important for a stabilized representation of the external world, but is also crucial for proper interactions with objects in the surrounding environment. So far, many studies have investigated how various depth cues affect size constancy in perception, but much less effort has been spent investigating how these cues affect size constancy in actions, such as grasping. Moreover, there is still some debate as to whether or not there is size constancy in grasping (i.e., whether or not people use the same grip aperture to grasp an object at different distances). Previous research in our lab showed that even patients with visual form agnosia exhibit excellent size constancy in grasping. This was true when the patients viewed the object binocularly, but not monocularly. A very recent study, however, demonstrated that grip aperture increases as viewing distance increases, even when participants viewed the object binocularly in a lit room with all environmental information available. We argue that the lack of size constancy in grip aperture reported in that study is the result of different distances between the hand and the target object when the viewing distance was manipulated. This change in hand-target distance might have introduced biomechanical differences, especially when grasping objects at a far distance. In the current study, we tested whether or not there is size constancy in grasping when this biomechanical confound is removed. To accomplish this, viewing distance was manipulated by changing the participants' head position while keeping the positions of the hand and object fixed. The first/closest distance was set at 15cm. The second distance was 1.5 times the first distance, and the final/furthest distance was 2 times the first distance. Stimuli consisted of spheres of three sizes: 2.5cm, 3.75 cm and 5 cm in diameter. Two extra sizes (1.25 cm and 6.25 cm) were also included but were not analyzed. Participants were asked either to grasp the sphere as quickly and accurately as possible with their thumb and index finger, or to manually estimate the diameter of the sphere using the same fingers. Participants showed excellent size constancy in both grasping and manual estimation (i.e. their grip apertures were unaffected by viewing distance), which suggests that grasping is governed by size constancy mechanisms when biomechanical differences are well controlled.

Disclosures: **J. Chen:** None. **R. Rinas:** None. **I. Sperandio:** None. **M.A. Goodale:** None.

Poster

623. Human Finger Movements

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Program#/Poster#: 623.16/UU10

Topic: E.04. Voluntary Movements

Support: Wenner-Gren Foundation Postdoctoral Scholarship

Title: Hand-held tools attenuate self-touch

Authors: *K. KILTENI, H. EHRSSON;
Karolinska Institutet, Stockholm, Sweden

Abstract: Human survival depends on the ability to perform quick and accurate movements with our limbs and with hand-held tools. To overcome the temporal delays and noise of the sensorimotor system, the brain uses internal forward models to predict the sensory consequences of actions as a function of motor commands (Miall and Wolpert, 1996; Franklin and Wolpert, 2011). Although it has been previously theorized that the brain forms forward models of hand-held tools (Imamizu et al., 2000, 2003; Higuchi et al., 2007; Imamizu, 2010; Imamizu and Kawato, 2012), direct experimental evidence supporting a link between tool use and predictions of forward models has been lacking. Specifically, whether these predictions are computed in the same way as those associated with limb movements without tools remains unknown. To address this question, we investigated whether the predictive attenuation of touch observed when touching one hand with the other (Blakemore et al., 1998, 1999; Shergill et al., 2003; Bays et al., 2005) would also be observed for touch applied with a hand-held tool. In Experiment 1 (n=12), we show that when the touch is applied with the index finger (no tool), it is attenuated only when the positions of the hands are aligned (distance between hands=0 cm) as during natural self-touch but not when the hands are placed at a distance of 25 cm ($t(71)=6.18$, $p<0.001$, CI=[0.29, 0.56]). In Experiment 2 (n=12), we demonstrate that self-touch with a hand-held tool (distance between hands=25 cm) is attenuated to the same degree as direct touch applied with the index finger when the hands are aligned ($t(71)=-0.40$, $p=0.687$, CI=[-0.25, 0.17]). Finally, in Experiment 3 (n=12), we show that touch is attenuated only when the tip of the tool is positioned so that it is aligned with the passive hand that receives the touch (distance between the tip and the hand=0 cm) but not when the tip of the tool is placed at a distance of 25 cm ($t(71)=11.14$, $p<0.0001$, CI=[0.69, 0.99]). In summary, the results demonstrate for the first time that the sensory consequences of actions involving hand-held tools are predicted and attenuated to the same extent –and under the same spatial principles– as actions involving the digits alone. Based on our findings, we propose that the brain uses a flexible strategy to predict the sensory consequences of movement: predictions are not made on the basis of the state of the body-part *per se* but rather on that of the *end-effector*, i.e., the digits during natural manual interaction or the tip of the tool during tool use. This computational mechanism allows hand-held tools to act as versatile natural extensions of the hands in terms of sensorimotor predictions.

Disclosures: **K. Kilteni:** None. **H. Ehrsson:** None.

Poster

623. Human Finger Movements

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 623.17/UU11

Topic: E.04. Voluntary Movements

Title: Quickness of load force production is limited by the quickness of grip force production

Authors: *M. UYGUR, L. FUNK;
Dept. of Hlth. and Exercise, Rowan Univ., Glassboro, NJ

Abstract: The ability to generate quick submaximal forces (i.e. neuromuscular quickness) is an important aspect of motor function that can deteriorate with aging or with the presence of neurological diseases. This function has been assessed through isometric force production tasks completed under instructions to produce each force pulse as quickly as possible to varying submaximal amplitudes. The slope of the regression line drawn between the peak values of brief force pulses and the peak values of rate of force development (named as rate of force development scaling factor; RFD-SD) quantifies the neuromuscular quickness. The R^2 obtained from the same regression line represents the robustness of RFD-SF as a controlled variable. To assess quickness, previous studies used ecologically valid tasks during which subjects were asked to grasp an instrumented cane-like device and to produce tangential force pulses (i.e. load force; LF) by pushing down on it. Although one has to produce a quick grip force (GF) pulse to generate the LF pulse in those conditions, only the quickness of LF was studied. Therefore, we do not know if the quickness of GF was a limiting factor for the quickness of LF. The aim of this study was to compare the RFD-SF and R^2 of GF and LF obtained in tasks when they acted together with those obtained in tasks when they acted individually. Twelve healthy young participants produced brief force pulses under three conditions: 1) gripping the GF-LF measuring device to produce both LF and GF pulses (GF-LF), 2) gripping the GF-LF measuring device to produce GF pulses (GF_{only}), and 3) pushing down on another device which was strapped to the subject's wrist to create LF pulses without using GF (LF_{only}). Results revealed a similar RFD-SF for GF and LF when they had to act together in GF-LF condition ($p>0.05$). However, RFD-SF for LF_{only} was higher than those obtained for LF in GF-LF condition ($p<0.01$) while RFD-SF values were similar between GF_{only} and those obtained from GF in GF-LF condition ($p>0.05$). Regarding R^2, results revealed similarly high values in all conditions with an exception of lower values obtained for LF in LF-GF condition. Overall, these findings reveal that quickness of grip force production could be slower than the quickness of load force production, suggesting the importance of quickness training of GF producing muscle especially in populations who are prone to falls.

Disclosures: M. Uygur: None. **L. Funk:** None.

Poster

623. Human Finger Movements

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Topic: E.04. Voluntary Movements

Support: NSERC grant

CIHR grant

Title: The impact of self-modulation of physiological tremor amplitude on its frequency components

Authors: *C. DUVAL[1,2], F. AYACHI[1], J.-F. DANEAULT[3];
[1]Sci. de l'activité physique, Univ. du Québec À Montréal, Montreal, QC, Canada; [2]Ctr. de recherche institut universitaire de gériatrie de Montréal, Montréal, QC, Canada; [3]Motion Analysis Lab. of Spaulding Rehabil. Hosp., Harvard, Boston, MA

Abstract: Aims: We have previously demonstrated that physiological tremor amplitude can be voluntarily modulated. However, what is not known is the impact of such modulation on the different frequency bands, and how it changes the time series characteristics in term of the nonlinearity and irregularity. We aim to determine the effect of modulation on physiological tremor characteristics. **Methods:** Finger physiological tremor (right hand) was recorded using a laser displacement sensor on 8 right-handed participants in a control condition and during a modulation of physiological tremor where they were asked to reduce the amplitude of their physiological tremor amplitude, without using co-contraction as a means to attempt to stabilize their finger. Each participant performed 3 trials of 45 s for the two conditions. Descriptors in temporal domain, RMS value, and frequency domain such as mean frequency (MNF), median frequency (MDF), the spectral index (FrI = MNF/MDF), high order central moments/statistic (SHOS) of the spectral density and power spectral entropy (SEn) were computed for the band-pass filtered signals to compare the two experimental conditions. **Results:** The results of the present study revealed significant differences, between the two experimental conditions, for RMS variable and the ratio *FrI*. We noted also a slight increase in MNF in the modulation condition compared to the control condition, indicating a slight shift of power towards high frequencies but this difference was not statistically different. The *SHOS* revealed a significant increase in the 2^{nd} moments (dispersion index) for the modulation condition, however, the spectral density shape remain asymmetric and leptokurtic during the two conditions. The *SEn* showed that spectral structures remained the same for the two conditions. Additionally, surrogate data test for nonlinearity, using time reversibility (*Tr*) and approximate entropy (*ApEn*), indicated that the recorded tremor signals had mostly nonlinear properties during both experimental conditions, but the signal during the modulation condition became more complex and irregular.

Using the Hilbert Huang Transform (EMD-HHT), we revealed a significant decrease of the amplitude for all intrinsic mode functions (IMFs) during the modulation trials, with important spectral shape alteration for the IMF-2 and IMF-3, which corresponded to the frequencies in the 7-11 Hz range associated oscillations generated within the central nervous system. **Conclusion:** These results suggest that voluntary tremor modulation will affect both the mechanical reflex and central neurogenic components of physiological tremor.

Disclosures: C. Duval: None. **F. Ayachi:** None. **J. Daneault:** None.

Poster

623. Human Finger Movements

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Program#/Poster#: 623.19/UU13

Topic: E.04. Voluntary Movements

Support: NIH TR000126

Title: Deficits in inhibitory force control in young adults with ADHD

Authors: *P. WANG[1], A. CHENNAVASIN[2], J. R. TUCKER[2], S. SAMIMY[1], M. REYNOLDS[1], C. HUANG-POLLOCK[3], K. A. NEELY[1];
[1]Dept. of Kinesiology, [2]Dept. of Biomed. Engin., [3]Dept. of Psychology, Pennsylvania State Univ., University Park, PA

Abstract: Attention-Deficit/Hyperactivity Disorder (ADHD) is a common, childhood-onset, heterogeneous neuropsychiatric disorder characterized by inattention and/or hyperactivity-impulsivity. ADHD persists into adulthood in up to 65% of individuals. In addition to the core behavioral features, motor impairments are reported in up to 50% of individuals and deficits in this domain may be associated with poor adaptive functioning in daily life skills, socialization, and self-direction. Although numerous studies have reported poor inhibitory control in ADHD, the button-press task that has been used in these studies records performance errors only via the presence or absence of a single key press. This all-or-nothing response makes it impossible to capture subtle differences in motor inhibition. In the current study, inhibitory control was measured using a continuous grip force task that provides a sensitive metric for understanding how inhibitory control is altered in ADHD. In this study, we used a classic go/no-go task as well as a grip force variant to measure motor inhibition in young adults aged 18 - 24 with (N = 40, 22 female) and without (N= 40, 22 female) ADHD. For each task, participants completed two blocks of 100 trials (25% no-go trials). The grip force task included two conditions: low (15% of maximal voluntary contraction) and high (60% of maximal voluntary contraction) amplitude.

Participants produced force for 750 ms with a 500 ms inter-trial-interval. In addition to the force task, participants completed a button-press task with an identical experimental timeline. As expected, adults with ADHD made more failed inhibits in the classic go/no-go paradigm, but also produced more force in no-go trials in both the low and high force amplitude grip force task. No between-group differences in mean force output were observed for go trials. Bivariate correlations revealed that the mean force produced in no-go trials was positively correlated with no-go failure rate for the classic go/no-go task, as well as self-reported measures of symptom severity on the Conners' Adult ADHD Rating Scales (CAARS-S:L), including Inattention/Memory Problems and Hyperactivity/Restlessness. This study provides a novel examination of motor output during inhibitory control. The results suggest that the current and most commonly used indices of inhibitory control are insufficiently sensitive to assess cognitive and motor impairments observed in adults with ADHD. Further, this work has important ramifications for studies examining the longitudinal course of the disorder.

Disclosures: P. Wang: None. **A. Chennavasin:** None. **J.R. Tucker:** None. **S. Samimy:** None. **M. Reynolds:** None. **C. Huang-Pollock:** None. **K.A. Neely:** None.

Poster

623. Human Finger Movements

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Topic: E.04. Voluntary Movements

Support: Wellcome Trust Institutional Strategic Support Fund Cross Disciplinary Awards, Cardiff University

REPAIR-HD European Union's Seventh Framework Programme 602245

School of Healthcare Sciences Strategic Development Fund, Cardiff University

Title: The development and validation of the moneybox test: a new measure of functional dexterity for people with huntington's disease

Authors: *S. P. CLINCH, M. BUSSE, M. LELOS, A. ROSSER;
Cardiff Univ., Cardiff, United Kingdom

Abstract: *Introduction:*
Loss of upper limb function is a common symptom in people with Huntington's disease (HD), a neurodegenerative disease that causes cognitive and motor abnormalities for which there is currently no cure. This can impact upon activities of daily living such as eating, getting dressed

and multi-tasking and can severely affect quality of life. As there is currently no sensitive upper limb outcome measure for people with HD there is an imperative for well defined, quantitative measures to track disease progression and to assess the impact of new therapeutic interventions. Here we describe the development and evaluation of the Moneybox test (MBT). This is a new functional dexterity multi-task assessment developed in accordance with translational neuroscience and physiological principles for people with a broad disease manifestation, such as HD.

Methods:

Participants with HD (n=30) and a healthy reference group (n=8) were recruited to perform the MBT whilst wearing small, non-invasive accelerometers on their wrists and chest, which were used to quantify involuntary movement. The MBT comprised 3 tasks, which required subjects to transfer tokens into a moneybox in order of size (baseline), value (dual task) with and without reciting the alphabet (triple task). This was tested alongside other well validated single and dual tasks including the Timed Up and Go and Letter Verbal Fluency, Stepping and the Stroop task, and Walking and Talking. Other measures specific to HD and quality of life were also recorded including the Unified Huntington's disease rating scale-total motor score (UHDRS-TMS), UHDRS-total functional capacity (TFC), Short Form-12 (SF-12) and the Problem Behaviors Assessment (PBA).

Results:

HD patients were grouped into stage of disease based on their UHDRS-TFC score. MBT performance was significantly reduced across every stage of disease compared to the healthy reference group. Uniquely the MBT significantly distinguished the subtle differences between the reference group and people with the earliest stages of HD. MBT performance significantly and more closely correlated with gold standard measures of impairment in HD such as UHDRS-TMS, UHDRS-TFC, SF-12 and PBA than any other single and dual outcome measures tested.

Conclusion:

The MBT provides a sensitive, affordable outcome measure for people with HD and is more sensitive across disease stage than other commonly used measures such as the Timed Up and Go task. Future work includes interpretation of accelerometer data to determine if involuntary movement increases with cognitive load in this disease population.

Disclosures: **S.P. Clinch:** None. **M. Busse:** None. **M. Lelos:** None. **A. Rosser:** None.

Poster

623. Human Finger Movements

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 623.21/VV1

Topic: E.04. Voluntary Movements

Title: Differential effects of left and right hemisphere stroke on a precision grip force task

Authors: B. KC, K. A. FERCHO, *L. A. BAUGH;
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Abstract: A common occurrence of stroke, especially a stroke involving the territory of the middle cerebral artery (MCA) is the presence of persistent motor impairments in the distal upper extremity contralateral to the lesion. Of particular importance is the ability to modulate fingertip forces used in grasping control, as this ability is crucial to the completion of many activities of daily living. Although previous research has shown deficits in fingertip force modulation in both the affected (contralesional) and non-affected (ipsilesional) hand, the causes for these deficits has not been adequately explored. An increasing body of research has elucidated the differing roles the left and right hemispheres play in motor control. Specifically, it has been suggested that the left hemisphere is critical for predicting effector and task dynamics, whereas the right hemisphere is involved in steady-state final position control. The presented research examined the ability for stroke survivors to generate forces using their index finger and thumb during a precision-grip isometric force matching task. We predicted that performance on the task would be reduced when compared to control participants when testing both the "affected" and "non-affected limb". Further, we predicted that performance between those stroke participants with right hemisphere damage (RHD) and left hemisphere damage (LHD) would be dissociable, due to the differing role each hemisphere plays in motor control. Thirty-five right-hand dominant stroke participants (17 with the Left-Hemisphere affected) with mild upper-extremity hemiparesis and 50 healthy, age-matched control participants produced forces of 5, 10, 15, 20, 25, and 30 percent of maximal voluntary contraction using their index finger and thumb. As expected, when compared to healthy control participants, both RHD and LHD stroke participants displayed deficits in the force matching task. Additionally, there were significant differences in performance metrics between those with RHD and LHD, with those with RHD showing increased difficulty with the task, regardless of the hand used. Taken together, these results provide further evidence of the distinct roles the left and right hemisphere play in motor control, while simultaneously highlighting the necessity to consider the differential contribution each hemisphere makes to best enhance post-stroke recovery of the fine fingertip forces required for successful object manipulation.

Disclosures: B. Kc: None. **K.A. Fercho:** None. **L.A. Baugh:** None.

Poster

623. Human Finger Movements

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 623.22/VV2

Topic: E.04. Voluntary Movements

Support: NIH TR000126

Social Science Research Institute

Title: Memory-guided force control in healthy older adults

Authors: *K. A. NEELY[1], S. L. BLOUCH[1], S. SAMIMY[1], A. CHENNAVASIN[2], M. REYNOLDS[1], N. DENNIS[3], M. DIAZ[3];
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Abstract: Visual feedback is a rich source of information for guiding human motor performance and provides a basis for the formation of memory to guide performance in the absence of visual feedback. The goal of this study was to examine visually-guided and memory-guided precision force in healthy young and older adults. Previous work demonstrates that healthy young adults decrease force output as a function of time when visual feedback is not available. We hypothesized that the rate of the decay of force output would be greater for healthy older adults. Two groups of participants, young adults (YA: N = 33, mean age 20.9 years) and older adults (OA: N = 34, mean age 69.4 years), completed 20-second trials of isometric force with their index finger and thumb, equal to 25% of their maximum voluntary contraction. The visual display contained a movable force bar and a stationary target bar. Participants were instructed to move the force bar vertically to overlap the target bar, which indicated the target amplitude. In the full vision condition, visual feedback was available for the duration of the trial. In the no vision condition, visual feedback was provided for the first 8 seconds of the trial. In the remaining 12 seconds, participants were instructed to maintain the same amount of force in the absence of visual feedback. For each visual condition, mean force for each second in the last 12 seconds of each trial was submitted to a 4 (trial) by 12 (time) by 2 (group) mixed model ANOVA. In the full vision condition, OA produced more force than YA; however, no other main effects or interactions were observed. In the no vision condition, OA produced more force than YA, and a time by group interaction was revealed. To examine this interaction, we calculated the rate of decay of force over the 12 seconds without visual feedback. An independent samples t-test demonstrated that YA had a faster rate of decay of force compared to OA. This finding suggests that OA may be better able to store and/or access motor memories to guide force in the absence of visual feedback.

Disclosures: K.A. Neely: None. **S.L. Blouch:** None. **S. Samimy:** None. **A. Chennavasin:** None. **M. Reynolds:** None. **N. Dennis:** None. **M. Diaz:** None.

Poster

624. Finger Movements: Physiology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 624.01/VV3

Topic: E.04. Voluntary Movements

Support: EU Erasmus mundus Joint Doctorate Programme MOVE-AGE (2011-0015)

Title: Cortico-cortical and corticomuscular (de-)synchronization in discrete movements: how does ageing affect inter-hemispheric interaction?

Authors: *P. BABAEEGHAZVINI[1], S. SWINNEN[2], A. DAFFERTSHOFER[1];
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Abstract: Many everyday tasks like tying your shoes require your hands to act in unison. Bilateral coordination, however, might be easier than moving hands or fingers independently. One reason for this could be an involuntary cross talk between hemispheres in the central nervous system. Cross talk may change as a function of age as, for instance, the integrity of the transcallosal fibers deteriorate. Yet it is unclear to what extent age-related structural changes yield a bias towards symmetry/asymmetry of bimanual performance and/or a lateralization/de-lateralization of cortical activity. To establish age-related changes in motor performance and in the cortical motor network, we compared 11 elderly (60-77 yrs) with 11 young participants (21-30 yrs) conducting bimanual coordination tasks with distinct degrees of difficulty. Participants controlled a cursor with left/right pinch-grip forces to follow a target. The degree of difficulty was manipulated by amplifying high-frequency components of one of the two forces when displaying the cursor - by this we sought to amplified the cross talk.
We recorded force production and defined the error of performance (E) via the distance between target and cursor. We also determined the Pearson correlation (ρ) and the F-statistics of Granger causality (G) between force traces as estimates of bimanual interference. All asymmetric tasks displayed increased E, ρ, and G rendering poor performance and strong left/right interference. The latter was stronger in the elderly as both E and F were larger than in the young group. E and F were positively correlated implying a Granger causal relation between left and right end-effectors to induce performance error.
Cortical and muscular activities were assessed through EEG and EMG, respectively. We reconstructed source activity using broadband LCMV beamformers with AAL-atlas-based regions-of-interest. Next to analyzing power changes, we focused on the amount of alpha and beta phase synchronization between bilateral M1, PM1, and SMA, and between M1 and contralateral finger flexors. In line with the behavioral outcome the asymmetric tasks revealed the most pronounced inter-hemispheric phase synchronization between bilateral M1 and PM1.

The bilateral phase synchrony discriminated the two groups: task-related synchronization was increased in the elderly. Cortico-muscular synchrony with the right first dorsal interosseous revealed a strong involvement of ipsilateral controllers only in the elderly. Taken together our results suggest an altered interhemispheric as well as cortico-muscular entrainment as prime cause for reduced dexterity of distal upper limb function in the elderly.

Disclosures: P. Babaeeghazvini: None. **S. Swinnen:** None. **A. Daffertshofer:** None.

Poster

624. Finger Movements: Physiology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 624.02/VV4

Topic: E.04. Voluntary Movements

Support: German Research Foundation (DFG) SCHE 1575/3-1

Title: Representation of finger movements from macaque area AIP, F5 and M1

Authors: *W.-A. SHENG, A. AGUDELO-TORO, H. SCHERBERGER;
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Abstract: The ability to move individual fingers is a specific feature of primates. In the macaque brain, the anterior intrapariental area (AIP), the ventral premotor area (area F5), and the hand area of primary motor cortex (M1) are strongly linked to the planning and execution of hand grasping movements. Whereas previous studies of individual finger movements have focused on execution signals in M1, we investigated here the population coding of all three areas for individual finger control. A macaque monkey was trained in a delayed finger task with visual cues, in which the thumb, index and middle finger were flexed and hold individually or in combination with its neighboring fingers (5 conditions). A micro-switch manipulandum detected flexion movements and provided tactile feedback. The task consisted of several epochs, in which “fixation”, “cue”, “go”, and “hold” were used for decoding. Floating microelectrode arrays were implanted in AIP, F5, and M1 contralateral to the moving hand (64 channels per area), allowing us to record spiking activity simultaneously from about 200 single and multi-units. Firing rates of individual units from all areas showed similar patterns: they were tuned for single and multiple finger movements (cluster-based permutation test, $p<0.05$) with different amplitudes. In particular, neural activities of combined finger movements were no simple linear combinations of individual finger activities. Comparing the tuning onset of the three areas, AIP units had a significantly earlier tuning onset at the population level than F5 and M1 (cluster-based permutation test, $p<0.05$), giving evidence of visual input before movement. Another way to

show the visual component of AIP was to calculate the partial correlation coefficient (pcc) between a type of error trial and its two types of corresponding correct trials ("cue error pcc" for the pcc between one type of error trial and the type of correct trial with the same cue, and "movement error pcc" for the pcc between the same type of error trial and the type of correct trial with the same movement). The cue error pcc in AIP was higher than the movement error pcc throughout the whole trial, while in M1 the movement error pcc exceeded the cue error pcc before hold epoch and reached maximum shortly after hold. The tuning onset and the pcc both supported the "visuomotor grasping circuit" hypothesis of Jeannerod. In conclusion, decoding of finger movement conditions (1 out of 5) was best for M1 (75 %), and significantly worse for F5 (59%) and AIP (47%) (ANOVA, $p<0.05$), suggesting that finger movement execution signals are best represented in M1.

Disclosures: W. Sheng: None. **A. Agudelo-Toro:** None. **H. Scherberger:** None.

Poster

624. Finger Movements: Physiology

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Program#/Poster#: 624.03/VV5

Topic: E.04. Voluntary Movements

Support: NIH R01 NS 046367

Title: Changes in the M2 projection to medullary precerebellar relay nuclei correlate with recovery of upper limb function after peri-rolandic injury in macaca mulatta

Authors: *W. G. DARLING[1], D. L. ROTELLA[2], M. A. PIZZIMENTI[2], J. GE[3], K. STILWELL-MORECRAFT[3], R. J. MORECRAFT[3];
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Abstract: There is considerable interest in the role of spared descending neural pathways following injury to cerebral cortex because these projections may play a role in recovery of upper limb motor control. Using high-resolution tract tracing we previously found that isolated lateral frontal motor injury (F2 injury) results in an enhanced corticospinal projection (CSP) from spared supplementary motor cortex (M2) residing in the lesioned hemisphere (McNeal et al., 2010 J Comp Neurol 518:568), but this favorable, upregulated response is unequivocally blocked following combined lateral frontoparietal injury (F2P2 injury) (Morecraft et al., 2015 J Comp Neurol 523:669). We used the same experimental cases (4 F2 lesions, 4 F2P2 lesions) and 3 control cases to determine if a similarly polarized neuroplastic response occurs in the

projection to medullary precerebellar relay nuclei, specifically the lateral reticular nucleus (LRN) and epi-olivary subnucleus (EO) of the LRN. We also examined whether the distribution of the projection to the LRN and EO was correlated with measures of recovery of reaching and fine hand motor function. We found that the M2 projection to the contralateral dorsal region of the LRN was enhanced in F2 lesioned monkeys, but was variably enhanced or weakened among F2P2 lesioned monkeys that appeared to be related to variations in F2P2 lesion size/location. The M2 projection to the contralateral EO was variably enhanced and weakened in both F2 and F2P2 lesioned monkeys. These altered M2 corticofugal projections correlated positively with recovery of both reaching to and grasping small food objects with the contralesional hand in F2P2 lesioned monkeys. However, recovery of grasping in F2 lesioned monkeys was also positively correlated with recovery of grasping but negatively correlated with recovery of reaching. Thus, changes in the M2 projection to precerebellar nuclei may favorably affect recovery mechanisms underlying contralesional arm reaching and hand fine motor function after peri-Rolandic injury. This may occur through a number of cerebellar output systems, including the cerebellar-thalamic-cortical feedback loop directed to spared M2 in the lesioned hemisphere.

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Poster

624. Finger Movements: Physiology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 624.04/VV6

Topic: E.04. Voluntary Movements

Title: Finger representations in sensorimotor cortex are not disrupted in musicians' dystonia

Authors: *N. EJAZ[1], A. SADNICKA[2], T. WIESTLER[2], K. BUTLER[3], M. EDWARDS[4], J. DIEDRICHSEN[1];
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Abstract: Musicians' dystonia (MD) is a neurological movement disorder that affects 1-2% of all professional musicians. It is considered a task-specific disorder that results in abnormal patterns of finger movements during musical performance. One leading hypothesis for the etiology of MD argues that repetitive movements result in abnormal representations of hand movements in the sensorimotor cortex (Byl et al., 1996). Specifically, the spatial distances of finger representations on the sensorimotor cortical sheet are thought to be reduced, resulting in

increased involuntary co-contractions during task-specific activity. Here, we use behavioral measurements and functional magnetic resonance imaging (fMRI) to determine whether sensorimotor representations are distorted in MD.
A cohort of professional musicians (N=8 without MD, N=9 with MD), were instructed to perform a finger individuation task with either hand. Participants made individuated force presses with an instructed finger at varying force levels, and the patterns of involuntary forces produced by the passive fingers of the instructed hand (enslaving), and by all fingers of the uninstructed hand (mirroring) were measured. In comparison to the control group, the MD group demonstrated increased enslaving ($t(16) = -3.15$, $p=0.006$) and mirroring ($t(16) = -2.978$, $p=0.009$) on both their affected and unaffected hands. Furthermore, similar to increases in magnitude, the patterns of enslaving were also different across groups ($\chi^2(1,6)=28.2$, $p<1e4$). Thus, in contrast to task-specific dystonic symptoms, these finger individuation deficits appear to be much more generalized.
We also probed the finger representations of the MD and non-MD group using 7T fMRI. Participants either made individuated force presses ('motor' condition) at low force levels or had single fingers passively lifted ('sensory' condition) inside the scanner. Although strong somatotopic organization was observed in the primary somatosensory cortex for both groups, we found no reduction in spatial distances as a result of dystonia in either the primary somatosensory (S1, $t(15)=-0.504$, $p=0.621$) or the motor cortex (M1, $t(15) = -1.468$ $p = 0.163$). Even when using more sensitive multivariate measures of pattern overlap, we found no significant difference in the arrangement of digit activation patterns between groups. This was true for both S1 and M1, and for both the motor and sensory task conditions.
To summarize, our results suggest that hand deficits in MD are partly generalized and not entirely task-specific, and furthermore challenge the notion that MD is caused by increased overlap of finger representations in the sensorimotor cortex.

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Poster

624. Finger Movements: Physiology

Location: Halls B-H

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Topic: E.04. Voluntary Movements

Support: OBI ONDRI Basic Science Program

Title: Theta burst stimulation primarily modulates motor cortex engagement for ipsilateral, not contralateral, finger movements

Authors: *J. A. MELTZER[1], A. FRANCOIS-NIENABER[1], B. BELLANA[1], M. HEBSCHER[1], T. DESCHAMPS[1], F. FARZAN[2];
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Abstract: Noninvasive brain stimulation (NIBS) is known to modulate cortical excitability, based on its effects on motor evoked potentials. Theta burst stimulation is considered either excitatory in its intermittent form (iTBS) and inhibitory in continuous form (cTBS). Many questions remain about the nature of these excitability changes: 1) whether they generalize to other regions of cortex, 2) how they affect task-induced activity in the stimulated region, and 3) how they affect distant regions, especially homologous areas in the contralateral hemisphere, which is highly relevant as several applications of NIBS are based on altering the balance of interhemispheric inhibition. To address these questions, we measured oscillatory neuronal activity before and after iTBS and cTBS, using magnetoencephalography (MEG). Specifically we studied the effects of left motor cortex stimulation on induced changes in oscillatory power associated with moving the left and right index finger.
12 right-handed participants performed a visually cued finger movement task, pressing a button with either hand. After ~25 minutes of MEG, participants underwent stimulation to left motor cortex, iTBS in one session, and cTBS in another. Next, they did another 50 minutes of the MEG finger movement task.
Finger movements induced the expected patterns of oscillatory reactivity in motor cortex - bilateral mu (8-12 Hz) and beta (15-30 Hz) power decrease, beta rebound, and gamma (65-85 Hz) increase, timelocked to movement onset. Beta rebound and gamma increase were strongly lateralized to left cortex for right-hand movements. Left hand movements, however, evoked bilateral activity in these measures.
The effects of TBS were mainly on activity associated with ipsilateral hand movements. Relative to iTBS, cTBS to left motor cortex induced increased gamma power in right motor cortex for right hand movements: i.e. inhibiting the dominant left motor cortex caused the right motor cortex to "come online" to support movement of the right hand. In contrast, the same stimulation *reduced* gamma power in the left motor cortex for left hand movements: i.e. inhibiting the left motor cortex reduced its involvement in left hand movements.
These findings suggest that NIBS may not have a strong effect on task activation in motor cortex for contralateral movements, suggesting that a similar level of activity is required to execute the movement regardless of the baseline state of excitability. Instead, NIBS may modulate the degree to which activity spreads to the other hemisphere. This provides empirical support to studies aiming to modulate transcollosal inhibition through NIBS, for example in stroke recovery.

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Poster

624. Finger Movements: Physiology

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Topic: E.04. Voluntary Movements

Support: NIAMS Grant R01 AR-050520

NIAMS Grant R01 AR-052345

Title: Task-dependent coherence across finger muscles

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Abstract: Multiple task-related muscles may be grouped together by the nervous system through the distribution of a shared control signal. This common intermuscular signal contains oscillations which may be useful in characterizing different neural control strategies. To date, the principles which determine the strength and frequency content of common oscillatory drive to groups of muscles are not well understood. In this study, we tested the hypothesis that the mechanical relationship between muscles, within the context of a given task, determines the spectral content of their shared neural drive. Ten healthy adults were asked to complete i) a static, isometric pinch using the thumb and index finger (2N force), ii) a sinusoidal, isometric pinch (1 to 3N force), and iii) rotation of a pinched object between the index finger and thumb of their self-reported dominant hand. Surface EMG signals were recorded from the first dorsal interosseous (FDI), abductor pollicis brevis (APB), and flexor digitorum superficialis (FDS). Coherence between the EMG signals recorded from each pair of muscles was analyzed (from 1 to 50 Hz) to determine if the frequency content of shared drive varied between muscle pairs. We found that during object rotation, both the FDI and APB muscles lost coherence (at nearly all frequencies) with the FDS muscle, while FDI:APB coherence increased at 10 and 40 Hz, and was unchanged at 20 Hz, relative to the isometric pinching tasks. During the sinusoidal modulation of pinch force, FDI:APB coherence was generally reduced compared with the other two tasks, while coherence between the FDI and FDS muscles (which are more directly synergistic) increased. In contrast, spectral power changed relatively little above 5 Hz across tasks. We interpret these findings as evidence that the spectral content of shared neural drive, as measured by intermuscular coherence, depends upon the mechanical contribution that each muscle makes to the task at hand, as well as the degree of function coupling required between muscles. We speculate that such dependence contributes to the known sensitivity of intermuscular coherence to movement and the dynamics of force production.

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Poster

624. Finger Movements: Physiology

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Topic: E.04. Voluntary Movements

Support: UoC EG CONNECT

Title: Differences in movement-related, inter-regional phase-locking in young and elderly healthy subjects

Authors: ***N. ROSJAT**[1,2], S. POPOVYCH[1,2], L. LIU[1,2], B. A. WANG[1], T. I. TÓTH[2], C. GREFKES[1,3], G. R. FINK[1,3], S. DAUN[1,2];
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Abstract: The vast majority of motor actions, including their preparation and execution, is the result of a complex interplay of various brain regions. Novel methods in computational neuroscience allow us to assess interregional interactions from time series acquired with in-vivo techniques like electro-encephalography (EEG). These methods provide different neuronal representations of movement (e.g. ERD, ERS, PLI). However, our knowledge of the functional changes in neural networks during non-pathological aging is relatively poor.
To advance our knowledge on this topic, we recorded EEG (64 channel system) from 18 right-handed healthy young participants (22-35 years, 10 female) and 24 right-handed healthy old participants (60-79 years, 12 female) during a simple motor task. The participants had to execute voluntary low frequency left or right index finger tapping movements.
We used the relative phase-locking value (rPLV) computed from the phases obtained by Morlet wavelet transformation of the Laplacian-referenced EEG data to identify the functional coupling of brain regions during the motor task. We analyzed the connectivity for electrodes lying above the left and right premotor areas (lPM: F3, FC3 and rPM: F4, FC4), supplementary motor area (SMA: Cz, FCz) and the left and right primary motor cortex (lM1: C3, CP3 and rM1: C4, CP4). We compared the resulting networks of significant phase-locking increase in time-intervals prior, during and after the movement.
Our analysis revealed an underlying coupling structure around the movement onset in the delta-theta frequency band (2-7 Hz), only. For young subjects, the connection from SMA to M1

contralateral to the moving hand showed a significant rPLV increase already in the preparatory phase of the movement. This synchronization remained significant during the movement and in a time intervalafter it. In elderly subjects, however, the change in rPLV between SMA and contralateral M1 was significant only during the execution of the movement. We furthermore monitored the behavioral performance of the two age groups and observed a lower movement speed in the elderly subjects. We therefore suggest that a lateralized rPLV between SMA and M1 prior the movement is needed to accurately initiate and perform the finger movements.

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Poster

624. Finger Movements: Physiology

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Topic: E.04. Voluntary Movements

Support: Uoc Emerging Group CONNECT

GR3690/2-1

GR3690/4-1

Title: Movement related intra-regional phase locking in the delta-theta frequency band in young and elderly subjects

Authors: ***L. LIU**[1,2], N. ROSJAT[1,2], S. POPOVYCH[1,2], A. YELDESBAY[1,2], B. A. WANG[2], T. TOTH[1], C. GREFKES[2,3], G. FINK[2,3], S. DAUN[1,2];
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Abstract: Motor actions are generated by complex interactions of various brain regions. The same brain regions can build various functional networks depending on the action to be performed. Identifying the neural signals that encode an action's components (selection, preparation and execution) remains a difficult task.
In the current study, we seek to identify common neural markers of movement execution valid for both age groups in cortical motor regions. Therefore we recorded electroencephalography (EEG) data from 18 young (11F, 22-35 years) and 24 elderly (12F, 60-78 years) right-handed healthy subjects as they were performing a simple motor task. The task required participants to

execute a left or right index finger button press triggered by a visual cue or by an uncued voluntary choice.
In both age groups, we found significant phase locking in the delta-theta frequency band (2-7 Hz) in motor areas contralateral to the moving hand prior to movement execution. This phase locking occurred irrespective of how the action was initiated (with or without a visual cue) and was not significantly different in strength between the two age groups. However, the length of phase locking, particularly in the primary motor area contralateral to the moving hand, was much shorter in the elderly than in the young subjects, meaning that phase locking occurred later and finished earlier in time.
In addition, we monitored the performance of the two groups of participants and observed that movement and reaction times were significantly longer in the elderly. Moreover, the maximum phase locking value around movement onset was, in the young subjects, correlated with movement time at the electrodes located above the primary motor cortex. This correlation between the electrophysiological effect and the behavioral performance shifted to frontal regions in the elderly subjects.
In summary, our results suggest that phase locking in the delta-theta frequency band, i.e. the enhanced intra-regional synchrony in motor regions contralateral to the moving hand, constitutes a prerequisite for triggering the movement execution. We furthermore hypothesize that the earlier the significant phase locking occurs the better the motor action can be initiated and performed. Finally, we also suggest that, in elderly subjects, the additional recruitment of the frontal regions might constitute a mechanism to support the execution of the motor task.

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Poster

624. Finger Movements: Physiology

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Marga and Walter Boll Foundation

Title: The frequency-specific modulation of ipsilateral activities in primary motor regions during preparation of two simple motor tasks

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Abstract: Task-dependent decreased powers over sensorimotor regions have been shown to be more widespread and less lateralized when it is close to the unilateral hand movement onset, which plays important role in the normal aging and recovery of the stroke patients. However, the mechanism underlying this change of less lateralization of powers is still largely unclear. In this study, we are interested in the mechanism by which the motor system drives the ipsilateral powers during motor preparation in order to keep the performance of movement under the control of two different initiating networks, namely, *Self-initiated* and *Visually-cued* actions. In order to answer our question, we collected 64-channel EEG data while participants performed self-initiated and visually cued button presses with their index fingers. We found a significant decrease of power in α-band (8-13 Hz) and β-band (14-30 Hz) over ipsilateral primary motor area (iM1) during movement preparation in both conditions. In *Self-initiated* condition, a significant spectral coherence between left M1 and right M1 was found in α-band for both hand movements. And, the higher LM1-RM1 coherence was associated with the more decrease of high-β (22-30 Hz) power over iM1. Additionally, we found a significant coherence between the supplementary motor area (SMA) and iM1 in the α-band prior to a left hand movement in the *Self-initiated* condition, which similarly regulates the high-β power over iM1. In *Visually-cued* condition, however, RM1 and premotor area (PM) has the significant coherence with iM1 in δ-θ-band (2-7Hz) only during left hand movement. Moreover, the higher coherence between the right PM and iM1 in δ-θ-band is correlated with more decrease of high-β power over iM1 during left hand movement. Our findings suggest that the ipsilateral β oscillations seem not merely the result of interhemispheric modulation, but involve additional task-dependent processing. Considered that the increased β oscillations are related with greater GABAergic inhibitory activity, we may conclude that the task-dependent control network is involved in the control of ipsilateral β oscillations by distinct neural control patterns, with the purpose of suppressing output to the muscles of the nonmoving hand from the ipsilateral cortex.

Disclosures: B.A. Wang: None. **S. Viswanathan:** None. **R.O. Abdollahi:** None. **N. Rosjat:** None. **S. Popovych:** None. **S. Daun:** None. **C. Grefkes:** None. **G.R. Fink:** None.

Poster

624. Finger Movements: Physiology

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 624.10/VV12

Topic: E.04. Voluntary Movements

Support: BBSRC Grant BB/N001370/1

Title: Activation of indirect pathways between ventral premotor cortex and the spinal cord by transcranial magnetic stimulation

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Abstract: In humans it is well established that the ventral premotor cortex (PMv) plays a key role in controlling grasping movement. PMv conveys grasp-related information to the primary motor cortex (M1), which converts it into a grasp-specific motor command relayed to hand muscles via the corticospinal pathway. However, monkey studies have reported that PMv, as well as having a number of indirect projections to the cervical spinal cord (e.g. via M1 and the brainstem), provides direct projections terminating in the upper and lower cervical segments. Whether such projections exist and are functional in humans is unknown. Here we used transcranial magnetic stimulation (TMS) over PMv to condition H-reflexes elicited via low intensity peripheral nerve stimulation (PNS) whilst subjects sat at rest. The TMS coil was placed over the caudal part of the inferior frontal gyrus (cortical surface MNI coordinates: X=57.4, Y=17.7, Z=15.7). PNS was applied percutaneously to the median nerve in order to elicit H-reflexes in the flexor carpi radialis muscle. In order to time the arrival of the descending and ascending volleys onto the spinal motoneurons accurately we measured the central conduction time (CCT; measured from M1) and peripheral nerve conduction time (PNCT) for each individual subject. H-reflexes were either elicited alone (baseline) or conditioned by PMv TMS. We investigated 6 inter-stimulus intervals (ISI), namely -4, -2, 0, 2, 4 and 6 ms, where negative ISIs indicate that PNS is delivered prior to TMS, 0 ms indicates that PNS and TMS-evoked volleys converge at the same time on the spinal motoneurons, and positive ISIs indicate that TMS was given prior to PNS. In addition, we tested 3 TMS intensities (80%, 100% and 120% of resting motor threshold). Our results show that while threshold conditioning pulses over PMv had no effect on H-reflexes, both subthreshold and suprathreshold pulses increased the H-reflex amplitude compared to baseline H-reflexes. Specifically, subthreshold PMv conditioning pulses significantly facilitated H-reflexes at -2 and 4 ms ISIs, whereas suprathreshold conditioning pulses only facilitated H-reflexes at a 2 ms ISI. To ensure these effects were not due to a spread of activation from PMv to M1, we also conditioned H-reflexes with TMS over M1. Our results allow us to speculate that recruiting sub- or suprathreshold PMv projections reveals a different time course of indirect interactions with upper and lower cervical segments. PMv projections have a net and diffused facilitatory effect on spinal excitability; with subthreshold outputs interacting with both upper and lower segments and suprathreshold outputs only with lower segments.

Disclosures: **K.L. Bunday:** None. **M. Pereira:** None. **M. Davare:** None.

Poster

624. Finger Movements: Physiology

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Topic: E.04. Voluntary Movements

Support: Marco Santello: NSF grant BCS-1455866

Andrew M Gordon: NSF grant BCS-1455865

Title: Digit position and forces covary during anticipatory control of whole-hand manipulation

Authors: ***M. MARNEWECK**[1], T. LEE-MILLER[2], M. SANTELLO[3], A. M. GORDON[2]; [1]Univ. Of California Santa Barbara, Santa Barbara, CA; [2]Teachers College, Columbia Univ., New York, NY; [3]Arizona State Univ., Tempe, AZ

Abstract: Theoretical perspectives on anticipatory planning of object manipulation have traditionally been informed by studies that have investigated kinematics (hand shaping and digit position) and kinetics (forces) in isolation. This poses limitations on our understanding of the integration of such domains, which have recently been shown to be strongly interdependent. Specifically, recent studies revealed strong covariation of digit position and load force during the loading phase of two-digit grasping. Here we determined whether such digit force-position covariation is a general feature of grasping. We investigated the coordination of digit position and forces during five-digit whole-hand manipulation of an object with a variable mass distribution. Subjects were instructed to prevent object roll during the lift. As found in precision grasping, there was strong trial-to-trial covariation of digit position and force. This suggests that the natural variation of digit position that is compensated for by trial-to-trial variation in digit forces is a fundamental feature of grasp control, and not only specific to precision grasp. However, a main difference with precision grasping was that modulation of digit position to the object's mass distribution was driven predominantly by the thumb, with little to no modulation of finger position. Modulation of thumb position rather than fingers is likely due to its greater range of motion and therefore adaptability to object properties. Our results underscore the flexibility of the central nervous system in implementing a range of solutions along the digit force-to-position continuum for dexterous manipulation.

Disclosures: M. Marneweck: None. **T. Lee-Miller:** None. **M. Santello:** None. **A.M. Gordon:** None.

Poster

624. Finger Movements: Physiology

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Topic: E.04. Voluntary Movements

Support: NIH Grant 1R01 HD057152

Title: Sensorimotor deficits in Carpal Tunnel Syndrome: Relation between electrodiagnostic and grasp behavioral measures

Authors: *P. J. PARIKH[1,2], W. ZHANG[4], K. GRIMM[3], M. ROSS[5], M. SANTELLO[2]; [1]Univ. of Houston, Houston, TX; [2]Sch. of Biol. and Hlth. Systems Engin., [3]Psychology, Arizona State Univ., Tempe, AZ; [4]Physical Therapy, The City Univ. of New York - Col. of Staten Island, Staten Island, NY; [5]Neurol., Mayo Clin., Scottsdale, AZ

Abstract: Carpal Tunnel Syndrome (CTS) is an entrapment neuropathy of the median nerve resulting in sensorimotor impairments in the hand. Damage of the median nerve in the wrist affects patients' ability to use tactile feedback from the affected digits, and therefore sensorimotor integration and overall hand function. Accurate quantification of these phenomena can potentially aid in CTS diagnosis and prognosis. The clinical diagnosis of CTS is based on electrodiagnostic tests (EDT), which is also used to assess CTS severity. However, in clinical settings the decline in manual dexterity is often determined using measures such as pinch strength and sensory acuity that fail to capture more complex and critical aspects of hand function, i.e., the ability of integrating tactile feedback with planning, selection, and execution of motor commands. Our studies on the effects of CTS patients on the control of grasping and manipulation revealed an impaired ability to generate, store, and retrieve sensorimotor memories from past manipulations, thus preventing planning and execution of multi-digit forces on a trial-to-trial basis. The present study quantified the extent to which CTS-induced deficits in sensorimotor integration (SI) and results of EDT correlate. We used data collected on CTS patients with mild (n=12) and moderate (n=17) severity from our previous studies to determine, using regression analysis, the correlation between EDT and SI measures. Subjects performed a grip and lift task that required them to lift an inverted T-shaped object using their thumb and four fingers. We considered grip force and the net moment exerted on the object measured at the time of lift onset as measures of SI. In moderate CTS cases, longer median sensory nerve distal latency (MSNDL) predicted larger magnitude of moment applied to the object at lift-onset ($p=0.03$). Larger moment on the object can alter its orientation, thus potentially affecting manual dexterity. No correlation was found between EDT and SI measures in mild cases. Our findings suggest that EDT measures in patients diagnosed with moderate CTS have a greater informational content as these measures, in addition to electrophysiological deficits, determine

the degree of sensorimotor integration deficits. Conversely, no correlation between these measures in mild CTS patients raises the question of whether they may capture very different, yet potentially complementary dimensions of hand function in the early stages of median nerve compression.

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Poster

624. Finger Movements: Physiology

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Topic: E.04. Voluntary Movements

Support: National Science Foundation BCS-1153034

Title: Learning and inter-limb generalization of novel visuomotor mapping with multiple fingers

Authors: *Q. FU, C. ZAMORANO, M. SANTELLO;
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Abstract: Human machine interfaces usually require users to learn new visuomotor mappings with multiple degrees of freedom. However, neural and biomechanical constraints may limit the extent to which new mappings can be acquired, stored, and generalized. To improve the design and training efficiency of these novel mappings, it is important to understand how the central nervous system exploits the available solution in motor space. We addressed this question through an experiment that required human participants (n = 5) to learn a novel visuomotor mapping with four finger forces. Specifically, participants had to control a 2D cursor in a center-out task by exerting grip force with the fingertips of their dominant hand while grasping a stationary handle. The finger forces were scaled to single finger maximum voluntary contraction forces for each subject, then mapped to cursor positions by a 4×2 matrix, such that the index, middle, ring, and little finger moved the cursor along +x, −x, −y, and +y axes, respectively. We used three experimental sessions that consisted of cycles of 36 targets which were evenly spaced around the starting area. Participants performed 12 cycles in the training session on the first day, and came back 24 hours later for a 2-cycle retention test session, and a 5-cycle across-hand transfer test session. Performance was measured by movement time and submovement characteristics for each target acquisition. With regard to movement time, participants started with non-uniform performance across quadrants. This indicates a differential initial force coupling of different combinations of fingers. After training, however, participants reached each

target at similar durations across all quadrants. We also found that there was no significant change in the duration and distance of submovements through training with a total of 432 targets, but the number of submovements per target decreased. Interestingly, we found that the percentage of submovements that were aligned with the direction of single finger actuation significantly increased, suggesting a control strategy based on sequential, rather than simultaneous, force production by individual fingers. Finally, we found that the acquired mapping can be well retained and generalized to the non-dominant hand on the second day. These preliminary findings provide new insights on motor skill learning with multiple degrees of freedom.

Disclosures: Q. Fu: None. **C. Zamorano:** None. **M. Santello:** None.

Poster

624. Finger Movements: Physiology

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Program#/Poster#: 624.14/VV16

Topic: E.04. Voluntary Movements

Title: Cortical activity reflects differential contribution of feedforward vs. feedback mechanisms during object grasp and manipulation

Authors: *P. MCGURRIN[1], J. FINE[2], K. SCREWS[3], M. SANTELLO[2];
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Abstract: Unique sensorimotor mechanisms appear to underlie manipulation using a grasp requiring self-selected (unconstrained) vs. predetermined (constrained) contact points. During unconstrained grasping, additional digit force corrections are required to compensate for variability in where the digits grasp the object relative to planned contact points. Our recent work demonstrated that a virtual lesion over primary motor cortex (M1) has a differential effect on the grasp execution depending on whether subjects used a constrained or unconstrained grasp. We believe that this is due to the different extent to which the sensorimotor system relies on feedback of digit placement to modulate forces. However, the offline M1 stimulation used in our previous work did not allow us to examine the corresponding spatial and temporal features of neural activation. Therefore, the present experiment used electroencephalography (EEG) to investigate activation in the larger grasping network during unconstrained and constrained grasping. Our hypothesis was that grasp type-dependent cortical activation would be associated with differential weighting of sensory feedback in the two grasp contexts. Six subjects performed a visually-cued grasp and lift task using a precision (thumb-index) grasp at unconstrained or

constrained locations. Trials were blocked such that the grasping context was similar across trials (blocked) or could vary from trial-to-trial (randomized). indicated by a visual ‘go’ cue on each trial In both contexts, we asked subjects to lift an object with an asymmetrical center of mass while minimizing tilt. Time-frequency analysis of EEG data revealed greater modulation in gamma band power (25-50 Hz) in the unconstrained than unconstrained grasping over left prefrontal and sensorimotor regions during the first 500 ms following the ‘go’ cue. Interestingly, this modulation was sensitive to whether subjects performed grasping trials in a blocked vs. randomized manner. Relative to the unconstrained context, increased gamma band activity was present in the constrained context during random trial presentation ($p < 0.05$), but decreased during blocks where the context was similar across trials ($p < 0.05$). Our findings are consistent with previous EEG work demonstrating changes in gamma power during motor memory retrieval tasks, and are the first to explore the involvement of specific frequency bands during grasping tasks requiring differential weighting of feedforward vs. feedback mechanisms.

Disclosures: **P. McGurrin:** None. **J. Fine:** None. **K. Screws:** None. **M. Santello:** None.

Poster

624. Finger Movements: Physiology

Location: Halls B-H

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Program#/Poster#: 624.15/VV17

Topic: H.02. Human Cognition and Behavior

Title: Theta and Beta-band activity reflects motor interference in dual-context sensorimotor adaptation

Authors: *J. M. FINE, D. MOORE, M. SANTELLO;
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Abstract: An issue in sensorimotor learning during object manipulation is understanding how previously learned motor commands can be generalized to manipulating the same object in a new context. Object manipulation requires combining visual cues provided by object geometry and implicit knowledge of object properties to select appropriate grasp points and digit forces. If visual geometric cues do not match object properties -e.g., visual symmetry and asymmetric mass distribution - initial manipulation yields large errors. Further improvement must rely on implicit recall of object properties to adjust motor commands. We previously found that the information learned from lifting an asymmetrically weighted object is not readily transferred to a new context, i.e., after a 180° object rotation. Large errors (object tilt) occur during transfer trials that are akin to, or larger than initial learning. Despite these results, errors made on transfer trials do not allow discrimination of whether or not this interference arises from treating the second

context as novel, or a bias induced by the previous manipulation. To distinguish between these interpretations, we used electroencephalography (EEG) to examine the neural dynamics of participants while they learned to grasp and lift a visually symmetric but asymmetrically weighted object. After an initial learning block, participants performed nine transfer blocks. This consisted of lifting the object once after having rotated it 180° (transfer trials), followed by rotation back to the original context for four trials. Consistent with our previous work, transfer and post-transfer trials exhibited differing degrees of switching interference (error). Initial learning involves selecting appropriate motor commands while overcoming the conflict between explicit visual cues and object dynamics that is learned implicitly. Conflict during action selection and response uncertainty has been linked with increased theta activity (4-8 Hz) over the medial frontal cortex (MFC), and beta (15 - 30 Hz) suppression over primary motor areas during response planning. Therefore, if the second manipulation context is treated as novel, we expected similar theta and beta activity in early initial learning compared to transfer trials. EEG data revealed that frontal theta and motor beta activity was larger during learning in the initial context compared to the rotated transfer context. This preliminary analysis suggests that motor errors during transfer arise from a motor command bias, which is likely due to use-dependent repetition from initial learning and an inability to switch contexts immediately.

Disclosures: J.M. Fine: None. **D. Moore:** None. **M. Santello:** None.

Poster

624. Finger Movements: Physiology

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Topic: D.06. Vision

Support: NSF Grant BCS-1153034

NSF Grant BCS-1152916

Title: Effects of visual cues of object shape and density differentially affect variability in anticipatory planning of digit placement and forces.

Authors: *T. LEE-MILLER[1], M. MARNEWECK[1], A. M. GORDON[1], M. SANTELLO[2]; [1]Biobehavioral Sci., Teachers College, Columbia Univ., New York, NY; [2]Sch. of Biol. and Hlth. Systems Engin., Arizona State Univ., Tempe, AZ

Abstract: Skilled object manipulation relies on precise anticipatory planning processes that in turn rely on visual estimates of object properties and prior grasping experience. The prevalent

theoretical framework about object manipulation is that sensory feedback plays a dual role: (1) acquiring information about object properties ('online' control), and (2) forming internal representations of object dynamics to anticipate the digit forces that will arise when manipulating objects ('feedforward' control). Visual information about an object to be grasped is used to select an appropriate grip type. A wide frontal-parietal cortical network plays a significant role for these visuomotor actions, in particular posterior parietal cortex being involved in planning of digit positions and forces during reach-to-grasp. Studies on anticipatory planning of object manipulation showed initial task failure (i.e., object roll) when visual object shape cues are incongruent with other visual cues, such as weight distribution/density (e.g., symmetrically shaped object with an asymmetrical density). This suggests that congruent and incongruent shape and density cues differentially influence anticipatory planning of digit forces and placement. We have recently shown differential modulation of digit placement and forces, but only when shape and density cues were congruent. When shape and density cues were incongruent, we found collinear digit placement and symmetrical force sharing. However, trial-to-trial variability in digit placement and forces were large. This is in line with the strategy that to generate a desired torque, a continuum of digit placement and force modulation may be employed. We tested whether shape and density cues would differentially influence the trial-to-trial variability of digit placement and forces during initial trials of a two-digit object manipulation task. Subjects grasped and lifted an object with the aim of preventing roll. In Experiment 1, the object was symmetrically shaped, but with asymmetrical density (incongruent cues). In Experiment 2, the object was asymmetrical in shape and density (congruent cues). In Experiment 3, the object was asymmetrically shaped, but with symmetrical density (incongruent cues). Results showed that even on unsuccessful trials, variability between digit placement and forces were correlated. Thus, though the congruency of visual shape and density cues affect overall outcome, the strategy employed is similar. This is in line with the theory of motor equivalence and influences our understanding of internal representations underlying planning and execution of grasping and manipulation.

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Poster

624. Finger Movements: Physiology

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Topic: E.04. Voluntary Movements

Support: Grant-in-Aids for JSPS Fellows 15J03233

James S McDonnell Sholarship Award

Title: Cortical representation of finger sequences in humans

Authors: *A. YOKOI[1,2], J. DIEDRICHSEN[1];
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Abstract: Understanding how the cortical motor regions control learned sequences of movements is one of the key issues in motor neuroscience. Here, using novel multivariate analysis techniques for human functional imaging data, we asked whether and how different areas along the hierarchy of motor and premotor regions represent different aspects of a sequence of finger presses, such as whole sequences, transitions between two consecutive presses, or individual finger movements.
We scanned the brain activation of 9 participants while they produce repetitive presses with one of the 5 single digits or one of 6 multi-digit sequences. All trials consisted of 6 finger presses executed at 4 Hz tapping speed. To determine to what degree the information was driven by control processes or by sensory re-afference, we also repeated all sequences using passive stimulation of the hand by replaying exactly the force patterns recorded during the previous active session.
To assess how these sequences are represented across different cortical regions we performed representation similarity analysis (RSA) on the local activity patterns in each region. We used a cross-validated distance measure, such that a systematically positive value indicates that the activation patterns for that pair of conditions were significantly different.
We found robust encoding for single finger movements in contralateral primary sensory and motor, as well as in dorsal premotor cortex, supplementary motor area, and parietal regions, both in the active and passive conditions. All areas also showed encoding for multi-digit sequences. However, this representation was only found for the active, but not for the passive condition. In primary sensory and motor cortex, the activation for each sequence was dominated by the pattern associated with the first finger in the sequence. In contrast, in premotor and parietal regions, the encoding for the multi-digit sequence was independent of the single-digit maps. These results suggest that premotor regions show a different neural state for each of the possible sequences. In

contrast, primary motor and sensory cortex receive a large descending input for the first finger press, after which the learned sequence can evolve using less synaptic activity.

Disclosures: **A. Yokoi:** None. **J. Diedrichsen:** None.

Poster

624. Finger Movements: Physiology

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Della Martin Foundation

Boswell Foundation

Title: Dexterous individuated finger representations for neural prosthetic applications in the anterior intraparietal cortex of a tetraplegic human

Authors: ***T. AFLALO**[1], C. ZHANG[1], N. POURATIAN[2], R. ANDERSEN[1];
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Abstract: The complexity of human and primate motor behavior is largely enabled by the dexterity of our finger movements. Work in neural prosthetics has demonstrated the feasibility of controlling the aperture of basic grasp types (e.g. power & precision grips) and while these studies are important steps towards empowering paralyzed individuals to perform activities of daily living, these basic grasp templates provide strong constraints on the neural prosthetic behavioral repertoire. Fine dexterous control of individuated finger movements is of recent evolutionary origin and is primarily mediated by the corticospinal tract. Other forms of hand behavior (e.g. hand-holds for climbing) can be controlled via other descending pathways. The anterior intraparietal cortex (AIP) in the posterior parietal cortex is a subregion of the cortical grasp network thought to emphasize transforming visual information about object shape into appropriate grasp plans, and similar to premotor cortex (F5/PMv), shows specificity for whole hand shapes suggesting a possible library of whole grasp actions. Given the distributed network controlling the hand, and a primary role in transforming visual information, it is unclear whether the functional properties of AIP are suitable for neural control of dexterous hand actions. We tested how populations of neurons in AIP coded individuated and combined finger movements and the suitability of these signals for closed-loop cortical control in tetraplegic patients implanted with Utah arrays for a brain-machine interface clinical trial. We found that individual

digit movements of the right and left hand are well represented. Some neurons were specific for a single digit while others were activated by several digits (e.g. right thumb and pinky, but not the index.) Functional overlap was prominent between matched digits of the right and left. Overlap was also prominent between the middle, index, and pinky potentially reflecting correlated motion during natural behavior and biomechanical factors. Digit representations coded for multiple degrees of freedom enabling decoding of 2D thumb movements. These neural representations provided a versatile set of control signals and could be used for a variety of prosthetic applications including the continuous control of individuated finger movements of a virtual avatar, two dimensional cursor control, and the detection of finger flexion movements for the neural control of a virtual piano and typing interface. Further, we found that the relationship between single finger movements and combined movements of multiple fingers and whole hand grasps was frequently complex, but with underlying structure.

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Poster

625. Gait: Aging, Injury, and Disease

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Topic: E.06. Posture and Gait

Support: NSF-GRFP

AHA Award #15SDG25710041

Title: Changes in perception of step length size after split-belt walking

Authors: ***C. J. SOMBRIC**[1], G. TORRES-OVIEDO[2];
[1]Dept. of Bioengineering, [2]Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Step asymmetry post-stroke significantly limits patients' mobility. It has been proposed that patients' reduced perception of their gait asymmetry contributes to their inability to recover symmetric walking (Wutzke et al. 2015). Thus, there is an interest in understanding if the perception of step asymmetry can be altered. A recent study indicates that split-belt walking, in which legs move at different speeds, changes the perception of asymmetric walking speeds in unimpaired subjects (Vazquez et al. 2015). Here, we hypothesize that the perception of asymmetric step lengths can also be altered after split-belt adaptation. To test this, we investigated subjects' perception of step lengths for each leg before and after split-belt adaptation. Participants (n=10, 25.2±4.3) first learned a spatial map of three distinct, subject-

specific, step length sizes (short, comfortable, and long) by observing their step length and the targeted step size. All visual feedback was displayed with an Oculus Rift and Vizard software. We assessed changes in subjects' perception of their step lengths after two walking conditions: normal walking (n=5) at 1m/s and split-belt walking (n=5) at 1.5 m/s (fast leg) and 0.5 m/s (slow leg). Step length perception before and after walking was evaluated by recording step length accuracy while subjects walked at 1m/s with reduced visual feedback projecting only 35% of the actual step length error. Both groups walked for 810 strides before step length perception was assessed. A catch trial (both legs walk at 1m/s for 10 steps) was introduced during the walking period to identify motor after-effects induced by the split-belt walking condition. A two-way ANOVA with two factors, (1) walking condition and (2) stepping leg, was used to test for difference in motor and/or perception after-effects across groups or legs. We found that subjects in the split-belt group had significant motor ($p=0.007$) and perception ($p=0.001$) after-effects compared to the control group. We also found that perception after-effects were distinct for each leg: the slow leg undershot the stepping target while the fast leg overshot it following split-belt walking. Importantly, all subjects maintained the step size spatial map since there were no differences ($p=0.66$) in stepping accuracy for the different targets tested without visual feedback before walking and after perception after-effects were extinguished. In sum, we found that split-belt walking induces changes in the perception of step lengths. This is important since paradigms like split-belt walking could be used to alter patients' active perception of limb position and improve their awareness of asymmetric stepping.

Disclosures: **C.J. Sombric:** None. **G. Torres-Oviedo:** None.

Poster

625. Gait: Aging, Injury, and Disease

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Topic: E.06. Posture and Gait

Support: NSF BRIGE 1342183

Title: Intertrial variability of EMG reveals lack of bilateral or inter-joint muscle synergies for walking in unimpaired and post-stroke patients

Authors: *P. A. ITURRALDE, G. TORRES-OVIEDO;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Activation of 'muscle synergies' has been proposed to underlie neural control of movement, and cortical damage is thought to change the structure of these neural commands

(Cheung et al. 2014). To test these hypotheses we identified muscle synergies in unimpaired and post-stroke subjects that were independent from task requirements. We specifically recorded 30 bilateral muscles in 16 chronic post-stroke subjects and 16 age and sex matched controls under two walking conditions imposing distinct movement demands: normal walking vs. split-belt walking, in which legs move at different speeds. We identified potential muscle synergies by computing covariance matrices indicating correlated activity across muscles. Importantly, we dissociated covariations in EMG signals due to task requirements and those due to common neural drive by analyzing 1) rectified and filtered EMG data ('full dataset') and 2) fluctuations in EMG recordings from the mean activity across strides ('intertrial dataset').
We found co-activations between anatomical (22/22 signif. correlations, median r^2=.73), multijoint (119/188, r^2=.30), and bilateral (149/225, r^2=.29) muscles in the full dataset, which have been previously interpreted as anatomical, multisegmental, and bilateral muscle synergies, respectively. However, only anatomical co-activations were observed in the intertrial dataset (22/22, r^2=.36), while the others became much weaker (r^2<=.08). We also found that muscle co-activations between bilateral muscles in the full dataset change across walking conditions to match changes in task constraints (median change in r^2 =.14), whereas muscle co-activations in the intertrial dataset were maintained the same.
Lastly, anatomical muscle co-activations identified in the intertrial dataset of patients were the same (22/22, r^2=.40) as controls and surprisingly symmetric across legs. Conversely, full dataset analysis revealed that stroke patients have less multijoint (94/188, r^2=.38) and bilateral (113/225, r^2=.30) muscle co-activations than controls, suggesting a deficit in patients task performance or reduced task demands (since all patients walked slower than controls).
Taken together these results suggest that only anatomical groups might receive unified neural drive, but correlated activity in muscles across joints and legs (multijoint and bilateral muscle synergies) reflects task demands, rather than shared neural control signals. As such, differences in multijoint and bilateral muscle synergies between patients and controls may represent patients' deficits in task performance or reduced task demands and not clear differences in neural commands.

Disclosures: P.A. Iturralde: None. **G. Torres-Oviedo:** None.

Poster

625. Gait: Aging, Injury, and Disease

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Title: Deficits in automatic postural responses are related to cerebellar involvement in people with Multiple Sclerosis

Authors: G. GERA[1], B. W. FLING[1], *F. B. HORAK[1,2];
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Abstract: Introduction: Balance problems are prevalent in people with Multiple Sclerosis (PwMS); however, little is known about the mechanisms behind such deficits. We aimed to investigate ability of PwMS to predictively scale their postural responses to gradually increasing magnitudes of discrete surface perturbations. Given the cerebellum is responsible for predictive scaling or feedforward control of postural responses; we hypothesized that deficits in scaling postural responses to increased perturbation amplitudes will be related to the involvement of cerebellum in PwMS. Methods: Subjects (24MS, 14Control) stood on a force platform that translated backwards in four blocks of predictable increasing amplitudes (3.6, 6.0, 8.4 and 12 cm). Each block consisted of five trials (total of 20 trials). To determine the role of feedforward mechanism, automatic postural responses to displacements was estimated as rate change of center of pressure under each foot before the feedback response could change the postural response. Predictive scaling was estimated by computing the slope of regression between early postural responses and gradually increased perturbation amplitudes. International Cooperative Ataxia Rating Scale (ICARS) was used as a clinical scale to assess the extent of ataxia. Diffusion weighted images (DTI) of brain were also acquired. Radial diffusivity (RD), an indirect neural marker of myelination, of cerebellar peduncles was calculated for each participant. Lower RD is interpreted as being indicative of better white matter tract microstructure. Results: To date, we have analyzed the data of 9 MS and 9 control subjects. Control subjects exhibited better scaling of postural responses to the increasing magnitudes of the postural perturbations than PwMS (Regression coefficients: Control: 0.75±0.38; PwMS: 0.37±0.65). For PwMS, slope of the rate change of center of pressure with respect to the perturbation magnitude was negatively correlated with the ICARS scores (r=-0.65). ICARS scores were also correlated to the radial diffusivity of the cerebellar peduncles (r=0.5). However, the radial diffusivity of cerebellar peduncles was not related to the rate change of center or pressure (Control: 0.12; PwMS: 0.3). Conclusion: Preliminary data suggests that deficits in predictive scaling of postural responses in PwMS seem to be related to the cerebellar involvement as evident by the relationship of ICARS and postural response scaling.

Disclosures: **G. Gera:** None. **B.W. Fling:** None. **F.B. Horak:** A. Employment/Salary (full or part-time): APDM. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); APDM.

Poster

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UL1 RR02414

U01 AG027810

U01 AG042124

U01 AG042139

U01 AG042140

Title: The effect of cognitive dual-task on balance and gait in older men

Authors: *P. CARLSON-KUHTA[1], A. LAIRD[2], M. MANCINI[1], E. S. ORWOLL[3], J. A. LAPIDUS[2], M. EL-GOHARY[4], F. B. HORAK[1,5];
[1]Neurol., [2]Sch. of Publ. Health, Biostatistics & Design Program, [3]Endocrinol., Oregon Hlth. & Sci. Univ., Portland, OR; [4]APDM, Portland, OR; [5]Res., VA Portland Hlth. Care Syst. (VAPORHCS), Portland, OR

Abstract: Background: Activities of daily living (ADL) require balance control, especially during functional movement while performing other attention-demanding tasks. Older people with limited information-processing capacity are often challenged when performing several tasks simultaneously, that require greater attention resources. Moreover, older people who perform poorly under dual-task conditions are at increased risk of falls. We utilized a group of elderly men who are part of the Osteoporotic Fractures in Men (MrOS) cohort, to examine the dual-task impact on gait and balance, using wearable inertial sensors. We hypothesize that the impact of a cognitive challenge would be larger on balance than straight ahead gait in older men.
Methods: Two-hundred seven, elderly men (mean age: 83, range 80-87) completed an

Instrumented Stand and Walk (ISAW) test, wearing inertial sensors (APDM, Portland, OR), added to their routine MrOS study visit at the Portland, OR site. The ISAW protocol consists of: stand quietly for 30 seconds, followed by walking seven meters, 180° turn, and walking back seven meters. The protocol was repeated two times – without and with a dual task of reciting serial subtractions by three's. Standardized response means (SRM) were used to determine relative dual-task effects on objective metrics characterizing balance (postural sway, turning and trunk motion in gait) and gait spatial/temporal metrics. An SRM value of 0.20 represents a small, 0.50 a moderate, and 0.80 a large dual-task response.
Results: Spatial and temporal measures of gait, such as gait speed, gait cycle time, stride length and angle at foot strike or heel off showed a large effect with the dual task compared to single task (SRM>0.8). Dynamic stability during gait, such as double support time also showed a large dual-task effect (SRM>0.8). In contrast, postural sway measures of amplitude, jerkiness, and velocity showed a small dual task cost (SRM<0.3), while sway frequency had a moderate dual task cost (SRM>0.5). Similarly, dual task cost was small when turning (SRM<0.2).
Discussion: We showed that a concurrent cognitive dual task affected spatio-temporal gait parameters more than balance during postural sway, gait or a postural transition, such as turning. This suggests that more attention is required for gait than balance by older men in this task. We plan to divide our participants into fallers and non-fallers (prospectively) to determine if the effect of a concurrent dual task on specific domains of mobility could potentially serve as a biomarker for future falls.

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Poster

625. Gait: Aging, Injury, and Disease

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Support: CSULB ORSP 2015-2016 Multidisciplinary grant

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Title: Biofeedback device for gait rehabilitation: a validity test

Authors: *K. A. LEYBA[1], I.-H. KHOO[2], P. MARAYONG[3], V. KRISHNAN[4], O. ROJAS[1], N. BALAGTAS[1];
[2]Electrical Engin., [3]Mechanical and Aerospace Engin., [4]Physical Therapy, [1]California State University, Long Beach, Long Beach, CA

Abstract: Stroke patients exhibiting gait asymmetry require rehabilitation to improve walking and reduce injury from fall. A device, called 'Walk-Even', which consists of force sensor embedded insoles, a wireless module, a microcontroller, and a laser trigger system has been developed which analyzes gait and provides auditory biofeedback to correct gait asymmetry in real-time. To test Walk-Even's accuracy in measuring the key gait parameters, an experiment was conducted on 17 healthy adults ages 18 to 28 to compare the measurements of Walk-Even with a commercial electronic pressure mat, the ProtoKinetics Zeno Walkway (Havertown, PA). During the experiment, participants wore Walk-Even while performing a straight walk at a self-selected pace on Zeno Walkway. Two conditions were tested: normal walking and simulated asymmetrical walking. Asymmetrical walking was simulated by attaching a 7-pound weight on the participant's ankle. Temporal gait parameters, including gait time (the time it takes for a person to complete one walking cycle), swing time (the time when the person's foot is off the ground), and stance time (the time when the person's foot is on the ground) were measured. The asymmetry ratio, defined as one minus the ratio between the stance time of the affected leg to the normal leg, was then calculated. Spatial gait parameters, including step length (the distance from a person's first foot contact to subsequent contact of the opposite foot), stride length (the distance from a person's first foot contact to next contact of the same foot), and average velocity were also determined.
From the experiment, data analysis will show if the correlation between gait time, swing time, stance time, and asymmetry ratio between Walk-Even and Zeno Walkway were good. [Data to be reported]. These results may indicate Walk-Even's ability to provide accurate gait measurements for future experimental use in stroke patient rehabilitation by providing biofeedback. Due to the affordability, portability, and user-friendly interface, Walk-Even could be a possible alternative to expensive commercial devices in analyzing gait asymmetry, and another option in traditional physical therapy treatment to correct gait asymmetry.

Disclosures: K.A. Leyba: None. **I. Khoo:** None. **P. Marayong:** None. **V. Krishnan:** None. **O. Rojas:** None. **N. Balagtas:** None.

Poster

625. Gait: Aging, Injury, and Disease

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VA Merit Grant I01RX001075-01.

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Title: The effect of external cues and closed-loop biofeedback on Freezing of Gait in Parkinson's disease

Authors: *M. MANCINI[1], G. HARKER[2], K. SMULDERS[2], J. G. NUTT[2], F. B. HORAK[2]; [1]Neurology, Balance Disorders Lab., Oregon Hlth. and Sci. Univ., Portland, OR; [2]Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Background. Accumulating evidence suggests that inadequate integration of sensory information and defective proprioceptive internal maps may underlie abnormal motor control in PD. Interestingly, freezing of gait most frequently occurs during tasks that require control of asymmetric motor tasks, such as turning or gait initiation and depend heavily on integration of proprioceptive information. For these reasons, augmenting somatosensory information with biofeedback during appropriate phases of the gait cycle may improve gait disturbances. Here we compare the effects of open-loop external cues (metronome) and closed-loop tactile biofeedback on freezing of gait (FoG) in Parkinson's disease (PD).
Methods. Twenty subjects with idiopathic PD with FoG (MDS-UPDRS III: 43±11 and new FoG questionnaire score: 18±7) performed a turning task, consisting of turning in place for one minute (changing turning direction after each full turn) while off their levodopa medication. Three inertial sensors were mounted on the posterior trunk and on each shin. Turning was compared across 3 randomized conditions: i) baseline (no cues); ii) turning to the beat of a metronome (control), and iii) turning with phase-dependent tactile biofeedback via light vibration to the wrists every time the ipsilateral foot was in stance phase. For each condition, a Freezing ratio calculated as the power spectral density ratio between high and low frequencies of shin accelerations and the percentage of time spent freezing during the task were measured. This study is in progress and will also examine 20 PD subjects without FoG.
Results. All subjects showed mild-to-moderate FoG during the assessment. At baseline, the Freezing ratio was 2.2±0.4, and it significantly reduced with both the metronome to 0.8±0.2 ($p<0.001$), and tactile-biofeedback conditions to 0.8±0.1 ($p=0.001$; one-way ANOVA $F=6$, $p=0.004$). Similarly, the % time spent freezing in the turning task significantly decreased from

45±5% at baseline to 18±4% in the metronome condition ($p<0.001$) and to 19±4% in the tactile biofeedback condition ($p=0.001$; one-way ANOVA $F=12$, $p=0.001$).
Conclusions. We observed a significant decrease in freezing of gait while turning in both a biofeedback (closed-loop) and externally-cued condition (metronome, open-loop). These preliminary observations suggest that augmenting somatosensory information with a phase-dependent biofeedback system relying on an unobtrusive modality, might be an effective tool in reducing FoG in everyday life.

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Poster

625. Gait: Aging, Injury, and Disease

Location: Halls B-H

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Program#/Poster#: 625.07/WW1

Topic: E.06. Posture and Gait

Support: CIHR (MOP-77548)

Title: The modulation of locomotor heading required for obstacle avoidance is altered by the presence of visuospatial neglect.

Authors: *G. ARAVIND, A. LAMONTAGNE;
McGill Univ., Montreal, QC, Canada

Abstract: Introduction: Altered perception of visuospatial information caused by visuospatial neglect (VSN) after a stroke; can lead to an impaired control of locomotor heading especially in response to visual stimuli present in the contralesional side of space. In a virtual reality scenario, we assessed how the presence of VSN influenced the ability of stroke survivors to modulate their heading in response to moving obstacles when walking towards a stationary target. **Methods**: Twenty-six stroke survivors (13 with and 13 without VSN) were instructed to walk towards a centrally-located target, while avoiding a collision with a moving cylinder that approached either ipsilesionally, contralesionally or head-on. Outcomes assessed were related to obstacle avoidance (onset-of-heading change, maximum mediolateral (MaxML) deviation, walking speed and target alignment (heading and head rotation errors with the target). Relationships between aforementioned measures with clinical measures of neglect severity were also examined. **Results**: Collisions with contralesional and head-on obstacles were observed in 75% of

individuals with VSN individuals compared to 38% of individuals free of VSN. In individuals free of VSN, walking towards the same side as the approaching obstacle emerged as a safer strategy while deviating to the side opposite to the obstacle seemed more demanding (e.g. earlier onset-of-heading change, larger MaxML deviations and faster walking speeds) and led to occasional collisions. Individuals with VSN deviated to the ipsilesional side for all obstacle conditions, hence displaying same-side and opposite-side strategies for ipsilesional and contralesional obstacles, respectively. In this group, collisions with the contralesional obstacles were frequent, invariantly observed for the opposite-side strategy, and were associated with large delays in onset of heading reorientation. Individuals with VSN further showed greater delays in the onset-of-heading change, smaller distances from the obstacle and larger errors in alignment with the target at the end of the trial, compared to individuals free of VSN. None of the locomotor outcomes were explained by clinical measures of neglect. **Conclusion**: VSN negatively affects the modulation of the heading in response to moving obstacles while walking, leading to frequent collisions with obstacles approaching from the contralesional side and from head-on. The locomotor strategies adopted by stroke survivors with VSN further resulted in a failure to align with the intended goal. These findings could explain the poor community ambulation abilities commonly reported in individuals with post-stroke VSN.

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Poster

625. Gait: Aging, Injury, and Disease

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Program#/Poster#: 625.08/WW2

Topic: E.06. Posture and Gait

Support: National MS Society PP-1506-04668

Title: Changes in dynamic balance and gait during challenging walking conditions in people with multiple sclerosis

Authors: *T. ONUSHKO[1], T. BOERGER[2], J. VAN DEHY[1], B. D. SCHMIT[1];
[1]Biomed. Engin., [2]Physical Therapy, Marquette Univ., Milwaukee, WI

Abstract: People with multiple sclerosis (MS) commonly have impaired balance control and often report that it is the most disruptive symptom of the disease. For example, people with MS have difficulty maintaining their balance when walking in new and unpredictable environments or when transferring their weight. These balance difficulties likely arise due to disorders of the sensory system that lead to inadequate motor responses during balance disturbances. As a result,

people with MS develop compensatory strategies to maintain balance during functional tasks. The purpose of this study is to understand the strategies people with MS use to maintain balance in a challenging walking environment. In this preliminary study, we recruited four people with MS (1 male) and asked them to walk on a treadmill mounted on top of a six-degree of freedom motion base system under 3 conditions: sinusoidal (0.12 Hz) medial-lateral (ML) translation (6 cm), combination of sinusoidal movement (0.12 Hz) in three-degrees of freedom (roll, pitch, yaw; RPY) (4 degrees), and no movement. We measured kinematics as subjects walked at their self-selected speed for 80 seconds for each trial. We analyzed step width, step length, cadence and margin of stability (measure of gait stability) during walking. Data are represented as percent change with respect to the stable walking surface. During the ML translation, MS subjects demonstrated wider (step width: +27.0%), shorter (step length: -3.4%) and faster steps (cadence: +3.1%), compared with unperturbed walking. During the RPY movement, MS subjects also demonstrated wider (step width: +8.1%) and faster steps (cadence: +2.0%), but had longer steps (step length: +4.0%). Margin of stability increased for both movement types, but the increase was greater for the ML translation (+9.0%) compared with the RPY movement (+2.1%). Previous studies have shown that a combination of increased step width and cadence, and decreased step length is a beneficial strategy for controlling balance during increasing frontal plane perturbations, which also increase the margin of stability. Our preliminary data suggest that people with MS have greater difficulty controlling their balance during more challenging walking conditions (i.e. RPY), and may indicate they are at greater risk for falls. This information could be used for rehabilitative interventions aimed at improving balance.

Disclosures: **T. Onushko:** None. **T. Boerger:** None. **J. Van Dehy:** None. **B.D. Schmit:** None.

Poster

625. Gait: Aging, Injury, and Disease

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Program#/Poster#: 625.09/WW3

Topic: E.06. Posture and Gait

Support: CIHR

Title: Auto segmentation of physical activities during a Timed-Up-and-Go (TUG) task in patients with Parkinson's Disease using a system of inertial sensors

Authors: ***S. BOGARD**[1], H. NGUYEN[2], K. LEBEL[3], P. BOISSY[3], E. GOUBAULT[2], E. LE[2], N. ROOFIGARI[2], C. DUVAL[2];
[1]Sci. of Physical Activity, Univ. of Quebec At Montreal, Montreal, QC, Canada; [2]Univ. of Quebec at Montreal, Montreal, QC, Canada; [3]Univ. of Sherbrooke, Sherbrooke, QC, Canada

Abstract: INTRODUCTION: Wearable sensors could provide clinicians with access to motor performance of patients with PD as they undergo physical and clinical rehabilitation by analyzing the quality of their movements. However, the data captured by these sensors often have to be manually segmented before analysis could be performed. This process can be time consuming and cumbersome. We recently proposed segmentation algorithms based on peak detection using inertial sensors to automatically segment common activities in daily living by identifying the transition points between tasks. These algorithms were developed using a population of healthy older adult; however PD patients often exhibited altered gaits and movements due to the neurological damages caused by the disease. The aim of this study was to investigate the transferability of these algorithms in segmenting these tasks in patients with PD. **METHOD:** A modified Time-Up-And-Go (TUG) task was used since it comprised of four common daily living activities; *Stand up*, *Walking*, *Turning*, and *Sit down*, all performed in a continuous fashion. Twelve older adults who were diagnosed with early PD (Y&H<=2) were recruited for the study. They performed three trials of a 10 meters TUG task. They were outfitted with 17 inertial sensors covering each body segment. Raw data from sensors were detrended to remove sensor shift, normalized, and band pass filtered in the frequency domain with an optimal frequency to reveal kinematics peaks that correspond to different activities. Segmentation was accomplished by identifying the time of the first minimum or maximum to the right and left of these peaks. Multiple sensors were used to detect the same task transition point to create a redundant system to prevent detection failure due to altered gaits in PD patients. The segmentation times were evaluated by comparing them with the times identified by two independent examiners who visually segmented these tasks. **RESULTS:** The results showed that the two examiners had a variance of 175±106 millisecond (*ms*) during visual segmentation and the automated algorithm had a variance of 317±138 *ms*. This demonstrated that the automation could reliably segment these tasks just as well as the examiners but in less time. This was further reinforced by the fact that, on average, the difference in the transition points identified by the examiners and the algorithm was 466±138 *ms*. The study also demonstrated the need for a system of redundant sensors to robustly adapt to the variability in the pathological gaits and movements in patient with PD which was absent in healthy older adults.

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Poster

625. Gait: Aging, Injury, and Disease

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Program#/Poster#: 625.10/WW4

Topic: E.06. Posture and Gait

Support: JSPS 16K16563

Title: Change in the cutaneous reflexes during walking in patient with chronic ankle instability

Authors: *G. FUTATSUBASHI[1,2], S. SUZUKI[1,3], H. OHTSUKA[4], T. KOMIYAMA[1,5];
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Abstract: Many patients with ankle sprain complain of loss of control of the ankle joint, which may be related to the frequent recurrence of ankle sprains. Ankle instability is usually strongly related to mechanical and functional degeneration, signifying that it may stem from sensorimotor or neuromuscular deficits. Furthermore, cutaneous reflexes and their regulation are also important aspects of sensorimotor control like as walking. We have previously shown that the suppressive middle latency cutaneous reflexes (MLRs: ~70–120 ms) of the peroneus longus muscle (PL) were more pronounced on the injured sides of patients with chronic ankle instability (CAI) than on their uninjured sides or in control subjects during tonic contraction of the test muscles during sitting. However, it remains unknown whether the MLRs of the lower limb muscles during dynamic condition (like as walking) are modified in the patients with CAI. Therefore, the present study investigated the cutaneous reflexes in the subjects with CAI during walking to shed light on the neural mechanisms underlying the regulation of the lower muscles. .
The participants were 10 individuals (8 males and 2 females) who had sustained CAI and age-matched control subjects. Participants were asked to walk on the treadmill at a speed of 4 Km/h. To elicit cutaneous reflex, non-noxious electrical stimulation of the sural nerve at the lateral malleolus was applied during treadmill walking (PT ×2.5, 333 Hz, 5 pulses). EMG signals were recorded from the tibialis anterior (TA), medial gastrocnemius (MG), peroneus longus (PL), vastus lateralis (VL) and biceps femoris (BF). Using the Force sensing resistors (FSRs) data, all responses for all data for each stimulus condition occurring in the same phase of the step cycle. Subsequence analysis was conducted on full-wave rectified and averaged reflexes in 16 equidistant phases of the movement cycle (n = ~20–30 responses per phase).
During treadmill walking, locomotor background muscle activation patterns in CAI were almost the same as those in the control subjects. In PL and BF, phase-dependent modulation of MLR was found in both CAI and control subjects following the sural nerve stimulation, though the degree of modulation were blunted in CAI. On the other hand, in the TA and MG, phase-dependent modulation of MLR was also found in both CAI and control subjects, but there was no significant differences.
These findings suggest that MLR in PL and BF in CAI subjects during walking was modulated, and would be relate to impairment of ankle joint regulation.

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Poster

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Topic: E.06. Posture and Gait

Support: National Multiple Sclerosis Society Grant PP-1506-04668

Title: Walking during continuous surface movement alters joint kinematics in persons with multiple sclerosis

Authors: *T. F. BOERGER[1], J. VAN DEHY[2], T. ONUSHKO[2], B. D. SCHMIT[2], A. S. HYNGSTROM[1];
[1]Physical Therapy, [2]Biomed. Engin., Marquette Univ., Milwaukee, WI

Abstract: Dynamic balance is believed to be impaired in persons with multiple sclerosis (PwMS), but has not been well described in the literature. Therefore, the purpose of our study was to characterize gait kinematics during continuous, sinusoidal movements of a walking surface. In order to perturb balance during walking, participants walked on a treadmill mounted atop a 6-degree of freedom motion platform (MOOG, INC). Six participants (age range 44-63, 1male/5 females) with MS walked at a self-selected speed during 4 sinusoidal movement conditions which were: no movement, pitch, roll, and roll, pitch, yaw combined. For movement conditions, we determined the maximum movement degree each participate could safely tolerate and perform our testing. Pitch and roll movements were performed at 0.12Hz, and the combined movement at 0.15, 0.16, 0.17Hz respectively. Retroreflective markers were placed on major landmarks to model sagittal plane lower extremity kinematics. We used a 14 camera Optitrak system and sampled the markers at 120 Hz to capture whold body motion. To quantify absolute variability of sagittal plane joint angles, we calculated the step-to-step standard deviation at each 1% of the gait cycle and then averaged each of these values across the gait cycle. Ankle angle variability increased from 2.28±0.76º during no movement to 4.61±1.29º, 3.67±1.02º, and 4.19±1.22º for pitch, roll, and combined respectively. Knee angle variability increased from 2.72±1.00º during no movement to 5.63±2.09º, 2.97±1.05º, and 4.73±1.93º respectively. Hip angle variability increased from 2.02±0.954º during no movement to 4.36±1.40º, 3.29±1.08º, and 3.84±1.48º. These data demonstrate that continuous walking movements require alterations in joint kinematics in PwMS. Therefore, our data suggest that our treadmill movements are more challenging than normal walking in PwMS. In the future, we will implement our movements into a training protocol designed to improve dynamic balance.

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Poster

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Topic: E.06. Posture and Gait

Support: Frederick C. Binter Parkinson's Disease Center

Title: Effects of Parkinson's disease on neural preparation and step initiation in unpredictable conditions

Authors: *R. E. POPOV[1], J. GAMACHE[2], J. R. HITT[2], J. T. BOYD[2], J. V. JACOBS[3]; [1]Neurosci. Grad. Program, [2]Univ. of Vermont, Burlington, VT; [3]Liberty Mutual Res. Inst. for Safety, Hopkinton, MA

Abstract: Objective: We sought to determine the effects of Parkinson's disease (PD) on cortical preparation and resulting motor execution during step initiation in predictable and unpredictable conditions.
Methods: Ten subjects with PD and 12 older adults without PD performed forward steps to a target in predictable and unpredictable conditions. All subjects received a warning cue followed by a cue to step two seconds later. In the predictable condition, subjects were instructed to step with a pre-defined leg before trials, and in the unpredictable condition, the instructed swing leg was presented with the cue to step. Scalp EEG was used to derive parameters of contingent negative variation (CNV) and beta event related desynchronization (bERD). Force plates under the subjects' feet were used to derive parameters of the anticipatory postural adjustment (APA). Passive-marker motion capture was used to derive step parameters. Mixed-model ANOVA were used to determine effects of group and condition on cortical preparation, the APA, and the step. Pearson's correlation coefficients were used to correlate clinical measures of PD severity to experimental task variables. Significance was set at 0.05.
Results: No significant differences in cortical preparation were detected between groups. Subjects with PD exhibited significantly less change between the predictable and unpredictable conditions in the number of trials with multiple APAs, largely due to higher values in the predictable condition. Across both conditions, subjects with PD also exhibited delayed time-to-peak APA and swing onset, diminished peak APA amplitudes, and prolonged APA durations as well as lower peak swing-foot velocities and smaller normalized step lengths. More impaired ambulatory capacity derived from the Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS) significantly correlated with later peak bERD (predictable condition) and APA amplitude onset latencies (unpredictable condition). Larger CNV amplitudes significantly correlated with larger normalized step errors (unpredictable condition) and greater motor symptom severity as recorded by the MDS-UPDRS (predictable

condition).
Conclusions: We confirm past reports of impaired step initiation with PD, which is similarly evident regardless of predictability of swing limb. Delayed cortical and postural preparation related to transitioning of motor state during step initiation associates with clinically impaired ambulatory capacity. Enhanced cortical preparation related to anticipation associates with impaired step accuracy and more severe motor symptoms.

Disclosures: **R.E. Popov:** None. **J. Gamache:** None. **J.R. Hitt:** None. **J.T. Boyd:** None. **J.V. Jacobs:** None.

Poster

625. Gait: Aging, Injury, and Disease

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

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Topic: E.06. Posture and Gait

Support: NIH 2R01 AG006457-29

K99HD078492-01A1

MRF ECI 316

Title: Motor switching during stepping in Parkinson's disease and freezing of gait

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Abstract: Introduction: Freezing of gait (FoG) is the strongest contributor to decreased quality of life in patients with Parkinson's disease (PD). Previous work has shown that FoG is associated with impaired set switching performance on cognitive tasks. Here, we evaluated motor switching in a stepping task in people with PD with and without FoG. We hypothesize that motor switching for step initiation is impaired in PD with FoG compared to PD without FoG.
Methods: Eighteen subjects with PD participated in this study, in their 'OFF' state. Nine subjects had self-reported FoG (Hoehn & Yahr 2, mean MDS-UPDRS-III 36) and 9 did not report FoG (Hoehn & Yahr 2, mean MDS-UPDRS-III 33). Subjects were instructed to take a step forward with their left or right foot depending on the auditory cue 'same' or 'switch', cueing a step with the same leg as in the previous trial or a switch to the contralateral leg. A total of 42 step trials were performed (21 of each condition). Angular velocity signals from inertial sensors placed at the shins were used to detect the instant of foot-off. Reaction time (RT) was calculated as the difference between onset of the auditory cue and the instant of foot-off.

Results: Reaction time between PD with and without FoG did not differ significantly. Contrary to expectation, the reaction times for 'switch' leg trials were faster than in the 'same' leg trials. This effect was similar for PD with and without FoG (no significant FoG x switch effect). In addition, switch costs in trials requiring switching to the most affected and to least affected side were not different. Accuracy on the task was high for both groups (>99%).
Discussion: Results did not support our hypothesis that PD with FoG would have higher motor switching costs for step initiation than non-freezers. In fact, in contrast to consistently reported delays in reaction time when switching between cognitive sets using upper limb responses, we observed *faster* responses when switching leg than when stepping with the same leg. Biomechanical and locomotor mechanisms related to balance control potentially underlie this discrepancy. For example, asymmetry in weight-bearing when returning from a step may be more advantageous to execute a next step with the contralateral leg, thereby masking central set effects. Alternatively, reciprocal pattern generators for locomotion may speed switching of steps between legs.

Disclosures: K. Smulders: None. **M. Mancini:** None. **F.B. Horak:** A. Employment/Salary (full or part-time): APDM. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); APDM.

Poster

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Topic: E.06. Posture and Gait

Support: Davis Phinney Foundation

Title: Alterations in upper and lower extremity kinematics in Parkinson's disease during dual-task conditions in a Two Minute Walk Test

Authors: *A. ROSENFELDT, A. L. PENKO, T. DEY, A. S. BAZYK, M. STREICHER, J. L. ALBERTS;
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Abstract: Introduction: Dual task (DT) conditions, or the simultaneous performance of two attention demanding activities, results in greater number of falls in those with PD compared to their healthy peers. Characterization of gait dysfunction during DT conditions is needed on a clinical and biomechanical level. The purpose of this study was: 1) To compare the distance covered in an over ground Two Minute Walk Test (2MWT) under single task (ST) and DT

conditions in individuals with PD; 2) To characterize changes in gait parameters observed during ST and DT conditions using a three-dimensional biomechanical gait analysis. Methods: Individuals with idiopathic PD performed the 2MWT on a flat, over ground surface under the following conditions: 1) Over ground walking alone ($2MWT_{ST}$); 2) While performing a cognitive task of Serial 7s ($2MWT_{COG}$); 3) While holding a cup of water ($2MWT_{MOTOR}$). The $2MWT_{ST}$ and $2MWT_{COG}$ were then performed on the Computer Assisted Rehabilitation Environment system, a virtual reality system with a fully integrated three-dimensional motion capture and a self-paced treadmill system. Results: Twenty-three participants completed the study. The mean UPDRS score was 33.0±13.1 with Hoehn and Yahr scores ranging between I-III. There was a significant difference in over ground distance walked between the $2MWT_{ST}$ (518.7 ± 85.2 ft.) and $2MWT_{COG}$ (432.3 ± 98.2 ft.) ($p<0.001$) and between the $2MWT_{ST}$ and $2MWT_{MOTOR}$ (467.7 ± 76.4 ft.) ($p=0.002$). During the $2MWT_{COG}$, individuals displayed a significant decrease in arm swing, velocity, cadence, step length, and a significant increase step width ($p<0.05$). Discussion: Individuals walked a significantly shorter distance during the 2MWT under DT conditions. The difference can be attributed to changes in velocity, cadence, and step length. By identifying gait parameters that are associated with DT losses, therapists may be able to provide more targeted treatments to improve gait performance.

Disclosures: **A. Rosenfeldt:** None. **A.L. Penko:** None. **T. Dey:** None. **A.S. Bazyk:** None. **M. Streicher:** None. **J.L. Alberts:** None.

Poster

625. Gait: Aging, Injury, and Disease

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Topic: E.06. Posture and Gait

Support: Dean's Innovation Award, Faculty of Medicine, Memorial University

Ignite Research Award

Research and Development Corporation

Canada Research Chairs Program

Title: Bipedal hopping reveals evidence of advanced neuromuscular aging among people with mild multiple sclerosis

Authors: *M. C. KIRKLAND, M. B. DOWNER, E. J. B. HOLLOWAY, E. M. WALLACK, E. J. LOCKYER, N. C. M. BUCKLE, C. L. ABBOTT, M. PLOUGHMAN;
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Abstract: Measures of walking such as the timed 25 foot walk test (T25FWT) may not be able to detect subtle impairment in lower limb function among people with multiple sclerosis (MS). We examined bipedal hopping to determine to what extent people with mild (Expanded Disease Severity Scale (EDSS) $\leq$3.5) MS would differ compared to age, gender and education-matched controls and elderly participants. We measured lower limb power (e.g. hop length, velocity), consistency (e.g. variability of hop length, time) and symmetry. Participants completed the T25FWT then walked at self-selected speed along an instrumented walkway (4.3m). After a rest, they then hopped using both feet four times along the walkway. We found that although the groups did not differ in self-selected walking speed and all groups scored below the six second cut-off for T25FWT, the Elderly group had significantly shorter hop lengths, more variability and more asymmetry than the Controls. The MS group were not significantly different from the Elderly or Controls in most measures and their values fell between the Control and Elderly groups. Hop length, but not measures of walking predicted EDSS score (R^2=0.38, p=0.02). Bipedal hopping is a potentially useful measure of lower limb neuromuscular performance.

Disclosures: M.C. Kirkland: None. **M.B. Downer:** None. **E.J.B. Holloway:** None. **E.M. Wallack:** None. **E.J. Lockyer:** None. **N.C.M. Buckle:** None. **C.L. Abbott:** None. **M. Ploughman:** None.

Poster

625. Gait: Aging, Injury, and Disease

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Topic: E.06. Posture and Gait

Support: NIH R21HD080398

KL2TR000152

UL1TR000128

Title: Decreased nonlinear dynamic gait stability in acutely concussed athletes: a longitudinal analysis of dual-task gait

Authors: P. C. FINO, C. W. SWANSON, *L. A. KING;
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Abstract: Dual-task (DT) gait deficits have been reported following concussion. It is unclear whether these DT deficits impact the risk of global destabilizations in everyday life. This ambiguity may be due to the relatively low ecological validity of previous DT paradigms or the use of traditional gait metrics. We examined the effects of several ecologically valid DT paradigms on the local dynamic stability (LDS) of gait following concussion over several weeks. Three tasks were chosen: reading, a congruent head turning task - participants responded to an auditory tone by turning their head towards the cue, and an incongruent head turning task - participants turned their head away from the cue. We hypothesized that individuals with a recent concussion would demonstrate a greater decline in stability with the addition of a DT compared to controls in each condition. Twenty-five college athletes with a recent concussion were tested longitudinally at approximately of 2, 5, 10, 16, 23, 30, 37, 44, and 51 days post-concussion. Twenty-five controls were matched and tested at similar intervals. Participants walked at their self-selected speed for two minutes with no secondary task and with each of the three DT paradigms. The audio cue was delivered randomly to either the left or right ear at inter-stimulus intervals between 2.1 and 3.6 seconds. Protocols and procedures were approved by the Oregon Health & Science University IRB. Data were recorded from lumbar-mounted inertial sensors. Lyapunov exponents calculated from the tri-axial accelerations estimated the LDS. A linear mixed model compared the effects of group, time, task, group*time and group*task interactions on the LDS during gait. A main effect of task was found with the reading task differing from normal gait ($p < 0.001$). No differences among normal, congruent or incongruent head turns was found. A significant group*task interaction was found for the reading tasks only ($p = 0.005$). The reading DT requires suppression of the vestibulo-ocular reflex to maintain visual fixation and elicited significantly less stability in the concussed group. The rate of reading did not differ between concussed and control groups demonstrating that the concussed athletes could perform the reading DT adequately. However, the decreased gait stability suggests less attentional allocation on stability or difficulty integrating the various senses used to maintain stability. This increased instability during a fairly common DT may increase their risk for re-injury post-concussion.

Disclosures: **P.C. Fino:** None. **C.W. Swanson:** None. **L.A. King:** None.

Poster

625. Gait: Aging, Injury, and Disease

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Topic: E.06. Posture and Gait

Support: Parkinson's Disease Foundation Pilot Grant

Title: Freezing of gait in Parkinson's disease consists of unique pressure-based gait phase transitions

Authors: *P. MAZZONI[1], D. ZANOTTO[2], F. MAGET[2], B. XU[1], S. AGRAWAL[2];
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Abstract: Freezing of gait (FOG) is a major debilitating symptom and cause of falls in many patients with Parkinson's disease (PD). FOG consists of sudden interruptions of the gait cycle of highly variable duration. It often does not respond to treatments given on steady basis, like medications, pacing sounds, and patterned visual backgrounds ("open-loop" interventions). We therefore plan to develop "closed-loop" interventions triggered by the onset of FOG episodes. This approach requires real-time detection of FOG onset. We introduce a new method of detection of FOG episodes based on underfoot pressure measured by custom-built instrumented footwear (SoleSound system). This fully portable system can measure temporal and spatial gait parameters and deliver auditory and vibrotactile feedback in response to those parameters. In this work, underfoot pressure data are recorded while a human subject walks freely in a 20-m range. We introduce a new variant of gait phase representation based on the binary on-the-ground/off-the-ground state of the heel and forefoot. We studied such representations of foot pressure patterns during freezing and non-freezing portions periods of walking in patients with PD and FOG. Analysis of gait phase transitions revealed unique reversals of transition directions at the onset of FOG episodes. These transitions were different from those observed during gait initiation, walking, and turning. This finding allowed accurate detection of FOG onset using a look-up table comparison of the present and previous two gait phases. This method is suitable for future implementation of rapid real-time detection of FOG, possibly as quickly as 500 ms after FOG onset. The unique signature of FOG in gait phase space may hold clues to the nature of the gait control abnormality that underlies FOG.

Disclosures: P. Mazzoni: None. **D. Zanotto:** None. **F. Maget:** None. **B. Xu:** None. **S. Agrawal:** None.

Poster

625. Gait: Aging, Injury, and Disease

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Topic: E.06. Posture and Gait

Support: NIH 2R01AG006457

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Collins Medical Trust

Medical Research Foundation of Oregon

Title: Callosal integrity and dynamic stability of gait in people with parkinsonism

Authors: *C. CURTZE[1], B. W. FLING[1], M. DALE[1], J. G. NUTT[1], F. B. HORAK[1,2]; [1]Oregon Hlth. & Sci. Univ., Portland, OR; [2]VA Portland Hlth. Care Syst. (VAPORHCS), Portland, OR

Abstract: Introduction
People with parkinsonism (PD) are at an increased risk of falls due to instability during walking. Impaired interhemispheric, communication has been suggested as a cause for decline in dynamic gait stability. Here, we studied the association of genu collosal integrity and measures of dynamic gait stability. The 'margin of stability' is a measure of how close a person is to instability, taking the spatio-temporal accuracy of medio-lateral foot placement with respect to the body center of mass into account.
Methods
We recruited 10 subjects with idiopathic PD, 10 subjects with frontal gait disorder, and 8 age-matched healthy controls. All subjects underwent diffusion tensor imaging (DTI) to assess white matter microstructural integrity through the genu of the corpus callosum. Gait was assessed using an instrumented walkway (GAITRite). Margin of stability was calculated as the minimal difference between lateral center of pressure (CoP) and extrapolated center of mass (XcoM) during gait. Subjects performed three trials of 8 meters at a comfortable walking speed.
Results
Significant correlations between genu fiber tract microstructural integrity (FA) and stride width (Spearman's rho -0.82) and margin of stability (Spearman's rho -0.77) were found for the subjects with frontal gait disorders but not for the idiopathic PD or healthy controls. Subjects with frontal gait disorders walked with a wider stride width and a larger margin of stability than subjects with idiopathic PD and healthy controls ($p<0.01$). The margin of stability increased with stride width (Spearman's rho 0.76, $p<0.001$). Coefficient of variation of margin of stability decreased with stride width in the Frontal and PD groups. Trunk displacement in the coronal plan was not different among the groups.
Conclusion
The inability for people with frontal gait disorders to tandem walk is related to mediolateral instability. By assuming a wide stride width, people with frontal gait disorders are more stable and less variable while walking than PD or control subjects with their normal gait width.

Disclosures: C. Curtze: None. **B.W. Fling:** None. **M. Dale:** None. **J.G. Nutt:** None. **F.B. Horak:** A. Employment/Salary (full or part-time): APDM. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); APDM.

Poster

625. Gait: Aging, Injury, and Disease

Location: Halls B-H

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Program#/Poster#: 625.19/WW13

Topic: E.06. Posture and Gait

Title: Executive function under dual-task conditions forecasts cognitive declines in older adults with mild cognitive impairment

Authors: *A. CLARK[1], C. CRADDOCK[2], R. WEST[2], H. KELLEHER[2], C. M. CULLUM[4], R. ZHANG[4], B. TSENG[3];
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Abstract: Background: Evidence suggests that older adults become more reliant on cognitive resources to compensate for the structural and functional declines in the brain as a result of natural aging. Moreover, simple motor tasks, such as walking, become more cognitively demanding over time. This paradoxical relationship between diminishing supply of cognitive resources and increasing attentional demand is exacerbated in people with Alzheimer's Disease (AD) and Mild Cognitive Impairment (MCI). The purpose of this study was to investigate the central hypothesis that the cognitive performance and manifestation of motor deficits under dual-task conditions is predictive of cognitive decline throughout adult lifespan.
Methods: Twenty-five middle-aged (MA) adults (7 males, 18 females, age=43±10.73 yrs, education=16±1.91 yrs), 23 older adults (OC)(7 males, 16 females, age 67±6.17 yrs, education=16±2.81 yrs), and 17 people with MCI (8 male, 9 female, age=64±5.24 yrs., education=16±2.00 yrs.) participated. Gait velocity at baseline was measured using a 10-meter walk test. Subjects were then asked to repeat the 10-meter walk under 4 different dual-task (DT) conditions designed to challenge their executive and memory function as they walk (i.e. DT-1: verbal fluency; DT-2: 5-digit span; DT-3: serial-7 subtraction; and DT-4: 3-item delayed recall tests). Gait velocity under each DT condition was recorded. One-way analysis of variance (ANOVA) was used to compare outcome variables across all groups.
Results: Both MA and OC outperformed the MCI group cognitively in DT-1, DT-2, and DT-3. More importantly, MCI patients produced less words per second in DT-1 relative to MA and OC ($p<0.05$). In addition, MCI patients also exhibited a more pronounced gait slowing than MA and OC in DT-4 ($p<0.001$).
Conclusion: These findings suggest that verbal fluency performance as measured by words produced per second under dual-task conditions, where cognitive function is challenged simultaneously when performing a motor (i.e. walking) task, is sensitive in differentiating early MCI patients who may appear cognitively intact. In addition, pronounced gait slowing under

dual-task conditions may also have the potential to serve as a behavioral marker to better identify MCI at its pre-clinical stage.

Disclosures: **A. Clark:** None. **C. Craddock:** None. **R. West:** None. **H. Kelleher:** None. **C.M. Cullum:** None. **R. Zhang:** None. **B. Tseng:** None.

Poster

625. Gait: Aging, Injury, and Disease

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Topic: E.06. Posture and Gait

Title: Educational level preserves cognitive integrity in older adults but not in patients with mild cognitive impairment

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Abstract: Background: Maintaining cognitive vitality at old age remains one of the most challenging endeavors in our rapidly aging society. It has been suggested that higher cognitive reserve (CR) may help maintain cognitive function later in life. However, with age, performance of routine motor tasks becomes more reliant on CR to compensate for the structural and functional declines in the brain. The purpose of this study was to examine the impact of education level on preserving cognitive performance in older adults. We hypothesized that people with higher education level will out-perform their counterparts in the dual-task (DT) tests where cognitive function is challenged simultaneously while performing a motor task (i.e. walking).
Methods: Sixteen patients (7 males, age=64±5, Education=15.9±2.1, BMI=26 ±3) with mild cognitive impairment (MCI), a prodromal stage of Alzheimer's disease (AD), and 23 non-MCI older adults (7 males, age=67±6, Education=15.8±2.9, BMI=27±4) participated. Educational level was documented (years). Gait velocity (m/s) at baseline was measured using a 10-meter walk test. Subjects were then asked to repeat the 10-meter walk under 4 different dual-task (DT) conditions designed to challenge their executive and memory function as they walk (i.e. DT1: verbal fluency; DT2: 5-digit span; DT3: serial-7 subtraction; and DT4: 3-item delayed recall &

sorting tests). Gait velocity under each DT condition was also recorded.
Results: No significant difference was found in educational level and body mass index (BMI) between 2 groups although MCI exhibited more gait slowing (21%) during DT4 than their non-MCI (5%) counterparts (p=<.001). More importantly, a negative relationship was found between educational level and gait slowing under DT4 in non-MCI subjects (r^2=.28, p=.01), indicating less gait slowing in those with more years of education. However, the same relationship was not found in the MCI group (p>.05).
Conclusion: Our findings suggest that years of education plays a protective role in normal cognitive aging as supported by the cognitive reserve theory. Interestingly, the same beneficial effect cannot be applied in the MCI group, suggesting that cognitive decline trumps educational level in MCI disease course.

Disclosures: R. West: None. **C. Craddock:** None. **A. Clark:** None. **H. Kelleher:** None. **H. Gaddam:** None. **C.M. Cullum:** None. **R. Zhang:** None. **B. Tseng:** None.

Poster

625. Gait: Aging, Injury, and Disease

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Topic: E.06. Posture and Gait

Title: Effects of long-term administration of the antioxidant resveratrol on age-related motor function deficits

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Abstract: Aging is associated with a loss of motor co-ordination that results in an increase in number of falls, greater incidence of hip fractures and a general decline in the quality of life of the elderly population. An increase in oxidative stress with aging, resulting in damage to dopaminergic neurons that play a role in controlling motor function, has been implicated in the onset of motor deficits. We hypothesize that antioxidant therapy will be effective in combating motor function loss induced by increase in oxidative stress. One such antioxidant is resveratrol, a phytoalexin found in red wine, grapes and berries. Resveratrol is known for its anti-inflammatory, anti-cancer and neuroprotective properties. Our goal is to elucidate the effects of resveratrol on motor deficits in an animal model of aging. We supplemented the diet of middle-aged (14-16 mo) C57BL/6 male mice with resveratrol (120mg/kg of food). The mice will be administered the diet chronically (6months) and will be tested for motor deficits every 2 months. Although we did not observe a significant improvement in exploratory behavior as measured by

the spontaneous activity test at an early time point (2 months), we postulate that the low bioavailability of resveratrol necessitates its administration for a longer time duration and will be effective in attenuating motor deficits following prolonged supplementation.
In addition to behavior changes, we also plan to elucidate the mechanisms through which resveratrol acts because this has not yet been fully understood. Extracellular protein kinase 1 and 2 (ERK1/2), belonging to the mitogen-activated protein kinases (MAPK) family, are involved in cell proliferation, differentiation, migration, and survival. Natural antioxidants, including resveratrol, have been known to activate these pathways *in vitro*. Therefore, we were therefore interested in testing the effects of resveratrol on these pathways in vivo. We have previously found that resveratrol activated ERK1/2 in the striatum of old mice (22-24mo) compared to young (2-4 mo) old mice following short-term resveratrol administration. We will therefore investigate if a chronic resveratrol diet activates these signaling pathways in the areas of the brain involved in modulating motor function (striatum and ventral tegmental area).

Disclosures: **S.D. Potdar:** None. **K. Sowa:** None. **J. Cavanaugh:** None.

Poster

625. Gait: Aging, Injury, and Disease

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Topic: E.06. Posture and Gait

Support: The Norwegian Health Association

Title: Changes in attentional control and gait in MCI and healthy elderly during Dichotic Listening in dual-tasking

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Abstract: Dual-task research explores the mechanisms behind fall risk in Alzheimer's disease and other dementias. However, investigations including mild cognitive impairment (MCI) are few. The present dual-task study, uses dichotic listening (DL) during walking to challenge gait and attention in MCI. Different auditory stimuli are presented to each ear. In three conditions, subjects forced their attention towards a specific ear, or freely attended stimuli from any ear. Thus, DL mimics real life situations that require different levels of attention during walking. Execution of DL and walking in a dual-task situation allows to study the interplay between motor control, cognition and perceptual integration in elderly populations with increased risk of

falling. **Aim.** Evaluate the effects of a new dual-task paradigm focusing on control of auditory attention on spatiotemporal gait parameters and cognition in healthy elderly and older people with MCI. **Methods.** 15 MCI subjects (*M* =72,87 years) and 15 age-matched controls, all right-handed. The experiment had four conditions: normal walking baseline and three dual-task DL-conditions: Non-forced (NF), Forced-Right (FR) and Forced-Left (FL). Gait measures of step length, stride length and speed were evaluated with the Optogait©-system. The mean and coefficient of variation (CV) were calculated and used for group comparisons for both feet and per foot to explore laterality effects. ***Statistics***. Mixed model ANOVAs with repeated measures were conducted. ***Results.*** MCI participants showed difficulty in attentional auditory control. In both groups, DL performance affected step and speed and their variability across conditions. All groups had shorter steps, decreased speed and higher variability that gradually become more accentuated from baseline to FL condition. Group differences were present as MCIs had higher variability, shorter steps/strides and were slower than controls. An interaction effect was observed in all groups on the FR condition where elderly and in special, the MCI group, had shorter strides and higher variability on the left foot. ***Conclusion***. Performance on DL during walking affects spatiotemporal parameters of gait in elderly controls and more evidently in MCI individuals. In particular, focusing attention to the right ear seems to disturb the contralateral lower limb. This finding needs to be understood as a disruption of the lateralization of motor control caused by competing sources of information. The use of DL during walking proves to be a useful paradigm to comprehend effects on gait caused by specific attentional constraints.

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Poster

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Topic: E.06. Posture and Gait

Support: CIHR Grant 325892

Parkinson Society Pilot Grant

Title: Higher levels of perceived groove in music improve spatiotemporal parameters of gait during accelerated rhythmic auditory stimulation

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Abstract: Music-based rhythmic auditory stimulation (RAS) is a cueing technique used in gait rehabilitation, typically involving synchronizing footsteps with the beat in music. The beat rate of the music is generally adapted to be at or slightly faster than the participant's walking pace. The effects of music-based RAS can be enhanced by using 'high groove' music (music that induces the desire to move), which elicits larger and faster strides than low groove music when walking at normal pace. However, RAS outcomes vary significantly across individuals, so other factors clearly remain to be optimized. One potential factor is whether participants are instructed to synchronize to the beat or not. Instructions to synchronize may be detrimental to participants who have difficulty perceiving a beat, resulting in higher cognitive demand, which is known to impair gait. Therefore, we examined how task instructions (synchronized versus 'free' walking) and beat perception abilities influence spatiotemporal parameters of gait when walking to high- and low-groove music. Healthy young adults (n = 37 to date, data collection ongoing) were randomly allocated to either synchronized or free-walking RAS. In both conditions, participants walked back and forth eight times across a sensor walkway to assess baseline gait parameters. Then, participants walked to high- and-low groove music that was adjusted to be 15% faster (beats per minute) than baseline cadence (steps per minute). Finally, participants completed the Beat Alignment Test to measure beat perception ability. Preliminary results indicate that RAS elicited longer, faster and more stable strides, but only when stimuli were high groove. In high groove conditions, stride velocity, cadence, and stride length significantly increased relative to low-groove music. Stride width decreased during high groove conditions, indicating greater gait stability. There also appear to be interactions between groove and synchronized versus free-walking, suggesting the effects of groove may be more prominent during synchronized than during free-walking RAS. Future analyses will investigate the relationship of these factors to beat perception abilities.

Disclosures: E.A. Ready: None. **L.M.J. McGarry:** None. **J.D. Holmes:** None. **J.A. Grahn:** None.

Poster

625. Gait: Aging, Injury, and Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

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Topic: E.06. Posture and Gait

Support: CIHR

Title: Auto detection of daily living activities during a Timed-Up-and-Go (TUG) task in patients with Parkinson's disease using multiple inertial sensors

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Abstract: INTRODUCTION: Recently, much attention has been given to the use of inertial motion sensors for remote monitoring of individuals with limited mobility, particularly in patients with Parkinson's disease (PD). However, the focus has been mostly on the detection of symptoms, not specific activities. Monitoring specific activities will allow us to analyze changes in patients' mobility during physical and clinical rehabilitation by characterizing the quality of their movements. We recently proposed detection algorithms based on peak detection using linear acceleration, angular velocity and angular displacement data from multiple inertial sensors in healthy older adults. Inertial sensors attached to different body segments were use to detect common activities such as: *Stand up*, *Walking*, *Turning*, and *Sit down* during daily living. However, PD patients tend exhibited pathological gaits and movements that are different from healthy adults. The objective of the present study was to test the feasibility and robustness of the recognition algorithm to detect common activities in daily living in patients with PD. **METHOD**: A modified Time-Up-And-Go (TUG) task was used since it comprised of these common activities all performed in a continuous fashion. Twelve older adults who were diagnosed with early PD (Y&H<=2) were recruited for the study. Participants performed three trials of a 5 and 10 meters TUG task. They were outfitted with 17 inertial motion sensors covering each body segment. Raw data from sensors were detrended to remove sensor shift, normalized, and band pass filtered in the frequency domain with an optimal frequency to reveal kinematics peaks that correspond to different activities. Specificity (true positive) and sensitivity (true negative) were used to evaluate the accuracy of the algorithm. **RESULTS**: Using the selected set of sensors, we were able to detect these activities with 100% sensitivity (n=216) during the 10 meters TUG. The specificity was also 100% for all tasks except *Sit down* (specificity=97%). During the 5 meters TUG, sensitivity was 100% for (*Stand up*, *Turning*, *Sit down*) and 99% for *Walking* (n=216). Specificity was 100% for *Sit down* and *Turning* and 97% for *Stand up* and *Walking*. The present study lays the foundation for the development of a comprehensive algorithm to detect activities in more naturalistic activities using *inertial sensors*, in hope of automating the analysis of motor performance within the detected tasks in patients with PD. Furthermore, our study show the need to have task specific sensor to accurately and reliably detect these tasks to minimize failure in population with altered gaits and movements.

Disclosures: H.P. Nguyen: None. **K. Lebel:** None. **P. Boissy:** None. **S. Bogard:** None. **E. Goubault:** None. **E. Le:** None. **N. Roofigari:** None. **C. Duval:** None.

Poster

625. Gait: Aging, Injury, and Disease

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Memorial University's Career Experience Program (MUCEP)

Title: Walking reveals compromised neural resource capacity during dual tasking in people with multiple sclerosis

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Abstract: Background: Cognitive-motor interference is prevalent in the everyday lives of people with multiple sclerosis (MS), often causing subtle impairment when attempting to undertake multiple tasks simultaneously (i.e.: Dual Tasking). Recently, MS studies have examined the effects of cognitive-motor interference by analyzing gait parameters, consistently showing a worsening of motor performance when paired with a cognitive component. However, the majority of studies have disregarded the potential cost inflicted on the cognitive task. We examined the effect that dual tasking has on cognitive performance on a mental tracking task (Serial 7's) in people with MS compared to healthy controls. **Methods:** Participant with MS (n=13; mean age ± SD, 43.92 ± 12.93) and age (±3 years), gender and education (±3 years) matched controls (n=10; mean age ± SD, 45.70 ± 12.90; (HC)). All participants performed the validated method of serially subtracting sevens as the cognitive component as both part of the Montreal Cognitive Assessment and later as a dual task while walking. The serial 7's task is a mental tracking task designed to test working memory and information processing abilities. Time elapsed as well as the number of correct/total answers was analyzed from audio records to determine a performance score. An algorithm of Mental Tracking Rate was created to negate the potential influence of a speed-accuracy tradeoff. **Results:** MS participants demonstrated a significant decline (52%), (from 4.15±1.34 correct answers to 2.15±1.99; $p<0.001$) compared to

HC, who did not significantly change between the two conditions (from 3.00±2.11 to 3.30±1.77; p=0.65). MS subjects provided significantly fewer *correct* answers than controls (4.62±3.66 and 10.40±8.45 respectively; $F(1,21)$=4.94, p=0.04). However, MS subjects and controls provided the same number of answers when walking 16m (MS group 6.69±4.03, HC group 11.30±8.12; $F(1,21)$=3.19, p=0.09) and spent the same time completing the task (MS group 39.63s±21.37, HC group 39.51s±13.76; $F(1,21)$<0.001, p=0.99). Mental Tracking Rate (% correct answers/min) correlated strongly with MS-related disability measured using the Expanded Disability Status Scale (EDSS; r(11)=-0.68, p<0.01). **Conclusion:** We propose that compromised mental tracking during walking in people with MS could be related to limited neural resource capacity and could be a potentially useful outcome measure to detect ecologically-valid dual tasking impairments.

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Poster

625. Gait: Aging, Injury, and Disease

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Topic: E.06. Posture and Gait

Support: NIH/NIGMS U54GM104942

NIH CoBRE P20GM109098

Title: Evaluation of locomotor phase modulation after cortical injury in the rodent stroke model

Authors: *K. TUNTEVSKI[1], S. FREEMAN[2], H. HU[3], X. REN[3], S. YAKOVENKO[4]; [1]West Virginia University, Centers For Neurosci., Morgantown, WV; [2]Neurosci., West Virginia Univ. - Centers For Neurosci., Morgantown, WV; [3]Dept. of Physiol. & Pharmacology, Exptl. Stroke Core,, Ctr. for Basic and Translational Stroke Research, West Virginia Univ., Morgantown, WV; [4]Exercise Physiol., West Virginia Univ. Sch. of Med., Morgantown, WV

Abstract: Stroke remains one of the most prevalent causes of mortality and morbidity in the world. The improvement of motor coordination in people with poststroke hemiparesis is one of the primary goals of rehabilitation. The functional reorganization of M1 after brain injury has been described by the idea of cortical vicariation, or the ability of surviving cortex to take over functions of the lesioned parts. This process has been directly studied with ICMS using mostly single electrode mapping techniques in monkeys and in rodents. Yet, the temporal dynamics and

the interplay between surviving neural pathways are poorly understood. The corticospinal dynamics that relies on complex processing in M1 and the integration of pyramidal and other descending signals on the spinal rhythmogenic elements need to be described. Our approach uses rats as an animal model due in part to their low variability in organization and location of M1 compared to higher mammals (e.g. cats and monkeys), and the smooth cortical surface that is well suited for MEA use. We have developed a behavioral task for rats that requires precise foot placement during locomotion on pegs (Tuntevski et al., 2016). Unlike in unrestrained overground locomotion where M1 cell activity is low (Drew et al., 2008), the peg walkway task is expected to require intact M to generated coordinated muscle patterns. Similar to stepping over obstacles in cats (Yakovenko & Drew, 2015), we expect that M1 in rats contributes to the regulation of flexor and extensor phases in the peg walkway task. Here, we tested the outcomes of mild cortical ischemic injury induced by a 60 min middle cerebral arterial occlusion (MCAo). Rats were briefly trained to walk on the walkway instrumented with the array of force sensors. Pegs were configured to produce either symmetric or two types of asymmetric locomotion favoring each side. After a week of recovery following MCAo, rats were performing the behavioral task for food rewards. The poststroke asymmetry in the phase control on the paretic side was measured with the asymmetry index calculated from the slopes in the relationship between stance and cycle duration in both forelimbs. The overstepping induced changes in the slope of the stance phase characteristic even in healthy locomotion, but it revealed injury lateralization in rats with MCAo. The slope of the stance phase characteristic was lower on the paretic side than on the 'healthy' side. This supports the hypothesis that M1 inputs may contribute to the coordination of muscle synergies in peg walking task in rats. Moreover, this method may provide the quantified tracking of precise limb recovery as a function of rehabilitation strategy after stroke.

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Poster

625. Gait: Aging, Injury, and Disease

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Topic: E.06. Posture and Gait

Support: The Michael J Fox Foundation Grant ID 9437

Title: Effects of cholinergic tone on gait and cognition in Parkinson's disease is the final title for the abstract

Authors: *D. MARTINI, B. W. FLING, M. MANCINI, A. GENDREAU, F. HORAK, J. NUTT;
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Abstract: Recent studies have demonstrated reduced levels of cholinergic tone in people with Parkinson's disease (PD), which has also been shown to be associated with reductions in gait speed and impaired postural control. The aim of the current study is to compare the effects of placebo and donepezil, a cholinesterase inhibitor, on measures of gait and dual-task cost in PD patients in a double-blind, placebo controlled, cross-over randomized clinical trial. Short-latency afferent inhibition (SAI), a physiological index of central cholinergic function, is being collected to determine if deficits in balance and gait correlate with reduced SAI. Finally, the attention network test (ANT) is being administered to determine if changes in gait are mediated by changes in attention. Data collection is ongoing and assessors remain blinded to group assignment. For this abstract, we present data collected from 24 participants with idiopathic PD at the end of their initial segment of the study (either drug or placebo). In addition to the ANT and SAI, we collected measurements of gait speed, percent time in double support, and stride time variability during Single Task (ST) and Dual Task (DT – serial subtraction by 3's) walking. Stride time variability during ST walking was significantly correlated with reduced SAI (rho = .44, p = .03), but not gait speed or double support time. During DT walking, no significant correlations were observed, however increased percent of time in double support (rho = .39, p = 0.06) and decreased gait speed (rho = -.38, p = 0.07) showed a notable association with reduced SAI. The relationships between ANT and SAI were nonsignificant. This preliminary data provides evidence that reduced cholinergic tone in persons with PD is related to impaired gait performance that may not be mediated by attention deficits. Our ongoing data collection will continue to investigate the potential utility of cholinergic manipulation as a strategy for treating balance and gait dysfunction in PD. The findings of this trial are intended to lead to more sharply focused questions about the role of cholinergic neurotransmission in balance and gait and eventually to Phase II B trials to determine clinical utility of cholinergic manipulation to prevent falls and improve mobility.

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Poster

625. Gait: Aging, Injury, and Disease

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Topic: E.06. Posture and Gait

Support: FRSQ Fellowship

Richard and Edith Strauss Fellowship

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Title: Goal-directed locomotion in post-stroke unilateral spatial neglect

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Abstract: ***Introduction***: Unilateral spatial neglect (USN), a highly prevalent post-stroke impairment, has been shown to affect the recovery of locomotion. However, our current understanding of goal-directed locomotion control in post-stroke USN is poor and existing literature lacks consensus on the expression of the deficits. Goal-directed reaching accuracy to remembered (i.e. off-line movement), as opposed to actual seen objects (i.e. on-line movement), is also more affected by USN, supporting the hypothesis of the ventral as opposed to the dorsal stream involvement in USN. Whether such distinctions exist for goal-directed walking remains to be explored. The objectives were to examine (1) goal-directed locomotion (mediolateral displacement [MLD] and heading errors [HE]); and (2) direction of deviation of the walking trajectory in subjects with and without USN (USN+ and USN-) and in healthy controls (HC). ***Methods***: Subjects (n=45) performed goal-directed walking trials to actual, remembered and shifting targets located 7 meters away at 0° and 15° right/left while immersed in 3-dimensional virtual reality scene viewed through an NVisor SX60 helmet mounted display. ***Results:*** The endpoint MLD and HE were greater in USN+ than in USN- and HC groups in all conditions. USN+ group showed greater MLD and HEs to left-sided targets, especially under remembered condition, compared to middle and right-sided targets. USN+ group demonstrated a trajectory deviation to the right (non-neglected) side for middle and left targets in all conditions. ***Conclusion***: In support of the ventral stream involvement hypothesis in USN, findings indicate that USN alters goal-directed locomotion to remembered targets, predominantly in the left hemispace. Moreover, results on the rightward deviation of the walking trajectory could help establish a consensus on the expression of USN. Goal-directed locomotion to remembered targets could also serve as a more sensitive assessment of USN than traditional paper and pencil tests.

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Poster

625. Gait: Aging, Injury, and Disease

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Topic: E.06. Posture and Gait

Support: NIH Grant 1R21HD076157-01A1

Title: Physical activity is more strongly related to gait variability while walking in a real-world environment than in a lab-based setting for community-living individuals post-stroke

Authors: *L. A. ZUKOWSKI[1], J. A. FELD[2], A. DREWS[1,3], D. FLETCHER[1,4], C. A. GIULIANI[1,2], P. PLUMMER[1,2];
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Abstract: High risk of falling, which is associated with high gait variability, may be contributing to low levels of community participation in stroke survivors. Gait variability is typically measured during undistracted walking in the clinic, and it is not known how gait variability relates to real-world walking and daily physical activity. The purpose of this study was to examine the relationships between amount of physical activity in the community and gait variability assessed in single- and dual-task conditions in the lab and in a real-world setting in adults post-stroke. **METHODS:** Sixteen ambulatory, community-dwelling adults with stroke (mean±SD: 49.9±14.0 years old, 15.8±10.7 months post-stroke) participated. Spatiotemporal gait parameters were recorded during single- and cognitive-motor dual-task walking in a lab and a real-world environment (hospital lobby). Coefficient of variation (CV, %) was calculated for stride length and stride time. Physical activity was recorded using a physical activity monitor worn for two consecutive days. Average walking bout duration (s), maximum walking bout duration (s), and total number of steps taken each day were calculated. Relationships between physical activity measures and gait variability were examined using Spearman's rho correlation coefficients. **RESULTS:** Maximum walking duration was the only variable related to stride time CV during single-task walking in the lab (rho=-0.50, p=0.05). Stride time CV during lab-based dual-task walking was not associated with any physical activity measures. Conversely, there were significant relationships between stride time CV assessed in the hospital lobby (single- and dual-task) and physical activity measures. Average walking duration (rho=-0.51, p=0.04), maximum walking duration (rho=-0.61, p=0.01), and total number of steps taken per day (rho=-0.51, p=0.05) were negatively related to stride time CV during single-task walking in the hospital lobby. Average walking duration was also negatively related to stride time CV during dual-task walking in the hospital lobby (rho=-0.67, p<0.01), notably stronger than the relationship with

single-task walking. None of the physical activity measures were related to stride length CV in any condition. **CONCLUSIONS:** Temporal gait variability during dual-task walking in the real-world is strongly related to amount of community ambulation after stroke, more so than gait variability measured in the lab, even under dual-task conditions. These data suggest that rehabilitation may need to target gait variability in high-distraction settings in order to improve physical activity in community-dwelling stroke survivors.

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Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

Location: Halls B-H

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH RO1 NS095366

Wings for Life

Title: Intrinsic properties and connectivity of spinal flexor and extensor rhythm generating neurons

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Abstract: The hindlimb locomotor central pattern generator (CPG) consists of ventrally located interneurons (INs) within the lumbar region of the spinal cord. Each limb contains a CPG with at least two layers, the rhythm generating (RG) layer and the pattern formation layer. The rhythm generating layer includes flexor RG INs, extensor RG INs, and inhibitory INs that connect and coordinate flexor-extensor RG INs ipsilaterally. Previous studies have proposed a flexor/extensor asymmetry where locomotor rhythmicity results from the activity of the flexor RG INs. The extensor RG INs fire tonically and are driven into bursting mode by the phasic inhibition from the rhythmically active flexor RG INs via inhibitory INs. This then suggests that there are differences in intrinsic excitability, burst generating mechanisms, and/or connectivity between flexor and extensor RG INs. Recently, Shox2 INs have been identified to be one of the cellular components of the rhythm generator for locomotion. Ablating these cells led to a reduction of the locomotor rhythm and no change in the flexor and extensor alternation. The goal of the present study is to determine potential factors contributing to a flexor/extensor asymmetry, focusing on

connectivity and intrinsic properties related to excitability and rhythmogenesis. We performed whole cell patch clamp recordings from identified Shox2 INs in reduced isolated spinal cord preparations from neonatal Shox2:Cre; tdTomato mice. Flexor and extensor RG INs were identified based on fluorescence and their preferred firing during drug-evoked locomotion *in vitro*. Voltage clamp and current clamp protocols were run to study the intrinsic passive and active properties of these cells, including ionic currents linked to cellular rhythmicity. Our data suggest that there are no differences in the cellular excitability between flexor-related Shox2 INs and extensor-related Shox2 INs. Potential rhythmogenic currents including I_h, persistent inward current, and T-type Ca^{2+} current are present in some of these cells. A differential distribution of these currents in the flexor/extensor Shox2 INs likely plays a role in establishing cell rhythmicity during locomotion. In addition to intrinsic properties, dual Shox2 IN recordings show that interconnectivity is scarce but may be preferential within flexor or extensor RG populations. Therefore, connectivity and expression of particular ionic currents in rhythm generating neurons may contribute to flexor-extensor asymmetry.

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Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH NINDS 1 R01 NS085006

Title: Intrinsic conductances of neuron types need not vary across animals

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Abstract: Identified neurons (types) can have intrinsic ionic conductances that vary several fold across different animals, which appear to be compensated by co-regulation of conductances to maintain stereotypical activity. Neuron types could also achieve this by having identical conductances across animals, although this has not been observed. Possibility of having identical conductances is even less likely in systems that exhibit substantial animal to animal variability in activity, such as the well-studied leech (Hirudo sp.) heartbeat central pattern generator network. A dataset of 6 leeches showed individual variations in a complete input-output network composed of firing patterns from all premotor inhibitory interneurons, strengths of their synapses

that impinge onto two bilateral pairs of output motor neurons, and those motor neurons' output firing patterns (Wright and Calabrese 2011, J Neurosci, 31:17555-71). Underlying motor neuron intrinsic conductances were estimated using a computational model that replicates recorded input-output properties. We expanded a previously used multi-objective evolutionary algorithm (MOEA) optimization method (Lamb and Calabrese 2013, PLoS ONE 8(11): e79267) to find the maximal conductance parameters of two Hodgkin-Huxley type, multicompartmental models of heart (HE) motor neurons in the leech ganglia 8 and 12, that match activity profiles recorded for each animal. This allows us to test the hypothesis whether intrinsic conductances necessarily differ across animals. The method failed to find matches to different activity profiles when we used average values of previously reported synaptic strengths. Matches were found only when we considered slight deviations in individual synaptic strengths. The newly found model synaptic strengths for each animal were not statistically different than the average synaptic strengths observed experimentally across animals. However, we have not quantified experimental variance of measured strengths to test whether the same is true on the individual animal level. We are improving our method of estimating synaptic strengths from spike-triggered averaging in voltage clamp recordings to encompass measures of variance (Norris et al 2007, J Neurophys 98:2992-3005). In conclusion, we found that varying intrinsic conductances cannot compensate for extrinsic network connectivity parameters even if they are only slightly wrong. Furthermore, with flexible synaptic strengths, we confirmed our hypothesis that a model neuron with same intrinsic conductances can replicate the substantial activity pattern variances in different animals.

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Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

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The Alfred Dunhill Links Foundation

Title: The role of the sodium pump in mouse spinal locomotor network activity

Authors: F. NASCIMENTO, L. D. PICTON, K. T. SILLAR, *G. B. MILES;
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Abstract: Locomotor rhythm generation must be flexible to adapt motor behaviour to prevailing demands. The Na^+/K^+-ATPase (or sodium pump) influences locomotor networks in *Xenopus* tadpoles (Zhang, Picton, Li, & Sillar, 2015, Sci. Rep. 5,16188) and *Drosophila* larvae (Pulver and Griffith, 2010, Nat. Neurosci. 13:53-9) in an activity dependent manner, but its contribution in equivalent mammalian networks is less well described. We describe a functional role for sodium pumps in murine locomotor networks. Using spinal cords isolated from neonatal mice (postnatal day (P)0-P4), we induced locomotor-related activity pharmacologically (NMDA 5μM, 5-HT 10 μM and DA 50μM) and recorded it through glass suction electrodes attached to lumbar ventral roots (L_1, L_2, L_5). The sodium pump inhibitor, ouabain (1-3μM), increased rhythm frequency and decreased burst amplitude, an effect that was more pronounced in the presence of 50μM DA. The sodium ionophore monensin (10μM) was used to increase intracellular sodium concentration and hence to mimic sodium pump activation. Monensin decreased locomotor burst frequency and disrupted right-left and extensor-flexor phase relationships. We next used dorsal root sensory stimulation in the absence of drugs in order to induce short episodes of more natural locomotor activity. When the interval between evoked episodes was reduced, episodes became shorter and slower. Ouabain (1-3μM) increased the frequency and duration of these episodes whilst monensin (10μM) exerted the opposite effect. These data suggest a possible role for sodium pumps in network self-regulation. Using spinal cord slices from neonatal mice (P2-P15) we performed whole-cell patch-clamp recordings from spinal motoneurons (MNs) and ventral horn interneurons (INs). We observed an ultra-slow afterhyperpolarisation (usAHP) in 40/97 MNs and 15/35 INs of ~5mV amplitude with ~60s duration following high frequency firing. In MNs, this was blocked by ouabain (1-3μM) and TTX (0.5μM), suggesting the usAHP involves a spike dependent increase in sodium pump activation. In MNs with a usAHP, monensin (10μM) caused a ~5mV hyperpolarisation. Perfusion of dopamine (10μM) increased the duration of this pump current by ~60%. Taken together, our data reveal a role for the sodium pump in mammalian spinal locomotor control circuits. Interestingly, this feature is present in some, but not all MNs and INs, suggesting that the functional role of the sodium pump may be restricted to specific neuronal subtypes, as in frog tadpoles (Zhang et al., 2015). The data also highlight the importance of the sodium pump as a spinal target for the neuromodulator DA.

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Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

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Title: PSD-95 protein expression in rat oro-maxillofacial motoneurons during postnatal development

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Abstract: Chemical synapses contain a number of diverse proteins, which form the postsynaptic density (PSD), and these are involved in synaptic structure, neurotransmission and signal transduction. PSD-95 is implicated in the formation and maturation of excitatory synapses. PSD-95 regulates the localization of NMDA receptors by means of binding with NMDA receptor subunit 2 (NR2). Rhythmical oro-maxillofacial activities, such as suckling and chewing, are generated in the brainstem, and we showed that NMDA receptors play a critical role in the rhythm and pattern generation and signal transmission around the trigeminal motor nucleus during prenatal and early postnatal development. Here, we immunohistochemically examined the temporal distribution of PSD-95 protein in developing rat brainstem from suckling to the mature chewing stage. There was early emergence of PSD-95 expression in the interneurons located in the medial region of the trigeminal motor nucleus. This observation supports the notion that the central pattern generator for rhythmical jaw movements is located peritrigeminal area.

Disclosures: K. Ishihama: None. **A. Tanaka:** None. **S. Honma:** None. **T. Yamanishi:** None. **T. Harada:** None. **S. Tanaka:** None. **A. Enomoto:** None. **H. Koizumi:** None. **S. Wakisaka:** None. **M. Kogo:** None.

Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 626.05/XX6

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSF Grant 1351129

Title: Spontaneous miniature outward currents are generated by calcium-induced calcium release in putative interneurons of the newt medullary reticular formation

Authors: *D. B. YAEGER, R. GREEN, L. MERLINO, R. RAPPOPORT, E. J. CODDINGTON;
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Abstract: Locomotor behavior of vertebrates is controlled by medullary reticulospinal neurons projecting to central pattern generators in the spinal cord.
Although reticulospinal neurons receive descending synaptic inputs from midbrain and forebrain areas, reticulospinal neurons also receive synaptic inputs from local interneurons. The properties of local interneurons in the medullary reticular formation have not been studied in any vertebrate, contributing to a gap in our understanding of how local connections regulate the activity of reticulospinal neurons. Using whole-cell recordings in brain slices made from adult rough-skinned newts (*Taricha granulosa*), we studied the properties of a class of putative interneurons. These interneurons exhibited spontaneous miniature outward currents (SMOCs), and the frequency of events increased with depolarization between approximately -55 and -45 mV. At potentials more depolarized than -45 mV, SMOCs disappeared and a sustained outward K^+ current was activated. SMOCs reversed polarity based on the Nernstian potential for K^+ and were reversibly blocked by tetraethylammonium. Furthermore, SMOCs were blocked when extracellular Ca^{2+} was replaced with cobalt, suggesting that SMOCs were generated by Ca^{2+}-gated K^+ channels in response to Ca^{2+} influx through voltage-gated Ca^{2+} channels. Furthermore, SMOC frequency temporarily increased and then decreased in the presence of caffeine, and gradually decreased in the presence of the Ca^{2+}-ATPase inhibitor cyclopiazonic acid, indicating that SMOCs depended on Ca^{2+} release from intracellular stores. Together these results indicate that SMOCs are generated by Ca^{2+}-induced Ca^{2+} release in putative interneurons. As SMOCs are activated at membrane potentials negative to spike threshold, these results indicate that SMOCs regulate spiking in putative interneurons in the medullary reticular formation.

Disclosures: D.B. Yaeger: None. **R. Green:** None. **L. Merlino:** None. **R. Rappoport:** None. **E.J. Coddington:** None.

Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: National Natural Science Foundation of China (Grant 31371104)

National Institutes of Health (NS066587, NS070583, and MH051393)

Title: Characterization of motoneurons and interneurons mediating rolling waves in *Aplysia* locomotor network

Authors: K. YU[1], D.-D. LIU[1], R.-N. JIA[1], Y. LOU[1], Y.-T. ZHENG[1], Y.-N. SU[1], S.-A. CHEN[1], T.-T. CHEN[1], W. YU[1], E. C. CROPPER[2], K. R. WEISS[2], *J. JING[1,2];
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Abstract: Rolling waves of neural activity are responsible for locomotion in a number of vertebrates and invertebrates. We study neural mechanisms underlying rolling waves in the Aplysia locomotor network. We previously identified a cluster of ~ 20 putative motoneurons on the ventral surface of the pedal ganglion. These neurons send their axons through nerves P1, P7, and P8 to the periphery, i.e., the foot, and exhibited different activity phases following tail nerve stimulation which elicits fictive locomotor programs as monitored by activity in parapedal commissural nerve (PPCN). We also identified interneurons that do not project out to any peripheral nerves.

Here, we use a neuromuscular preparation to demonstrate that stimulating a substantial fraction of this cluster's neurons elicits foot-muscle contraction in a frequency-dependent manner, thus indicating that many of them are motoneurons. After tail nerve stimulation, phasic activity of these motoneurons is associated with rhythmic contraction of the foot muscle and PPCN bursting activity.

We further analyzed the phasic activity of this cluster of motoneurons relative to PPCN bursting activity following tail nerve stimulation. If the phasic activity of a motoneuron precedes the PPCN activity for a full cycle, we define the relative phase as -100%. If the phasic activity of a motoneuron overlaps with PPCN activity, we define the relative phase as 0%. We then recorded nearly all of the neurons in this cluster in single preparations (n = 5). Using the above definitions, we found that the distribution of relative phases of these motoneurons is largely continuous, covering from -100% to 0%. However, we also found that there tend to be more motoneurons at two time periods: -40% to -60% and -10% to +10%, suggesting that activity of motoneurons may also be partially clustered. This implies that rolling movements may also occur in bouts. In addition, we found that motoneurons with similar relative phasing are more likely to be electrically coupled than motoneurons with different relative phasing. Finally, we found that several interneurons show relative phasing of about -69.2%, and that these are usually electrically coupled to motoneurons with slightly delayed relative phasing, e.g., about -57.8%, suggesting that interneurons may provide a driving force for the rolling wave.

In summary, we have demonstrated that a cluster of motoneurons may contribute to the formation of rolling waves in Aplysia. In addition, electrical coupling between specific motoneurons, and between motoneurons and interneurons may be one of the important synaptic mechanisms underlying rolling waves.

Disclosures: K. Yu: None. **D. Liu:** None. **R. Jia:** None. **Y. Lou:** None. **Y. Zheng:** None. **Y. Su:** None. **S. Chen:** None. **T. Chen:** None. **W. Yu:** None. **E.C. Cropper:** None. **K.R. Weiss:** None. **J. Jing:** None.

Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

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Program#/Poster#: 626.07/XX8

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Intramural Program of NINDS, NIH

Title: Optogenetic analysis of the role of motoneurons in the regulation of locomotor-like activity in the neonatal mouse spinal cord

Authors: M. FALGAIROLLE[1], J. G. PUHL[2], A. PUJALA[3], *M. J. O'DONOVAN[1]; [1]NINDS, NIH, Bethesda, MD; [2]Univ. of Minnesota, St. Paul, MN; [3]Janelia Farms Res. Campus, Ashburn, VA

Abstract: Stimulation of motoneuron axons in an isolated preparation of the neonatal (P0-4) mouse spinal cord can activate the central pattern generator (CPG) for locomotion. To establish if motoneuron firing can influence the CPG during locomotor-like activity evoked by drugs, we generated mice in which either ChannelRhodopsin-2 (ChR2) or Archaerhodopsin-3 (Arch) was expressed in $ChAT^+$ neurons. We used an LED system to illuminate the cord over the lumbar (L1-L6) or more restricted segments. In ChAT-ChR2 mice, a train of light pulses at 1-4 Hz for 10 sec activated locomotor-like activity in the absence of drugs confirming our earlier experiments with ventral root stimulation. Arch-ChAT mice were used to examine the effect of silencing motoneuron firing on the locomotor rhythm recorded from slow ventral root potentials (after low pass filtering the neurograms) and from whole cell recordings from motoneurons. Individual extensor motoneurons were hyperpolarized by 5-20 mV during illumination and their firing was abolished. Firing was easier to suppress in extensor compared to flexor motoneurons, and in the L5/6 roots compared to the L1/2 roots. This appeared to be because the primary rhythmic synaptic drive to flexors is excitatory and that to the extensors is inhibitory. Illumination frequently hyperpolarized extensor motoneurons below the chloride equilibrium potential rendering their inhibitory synaptic drive depolarizing and in phase with flexor activity. Suppression of extensor bursting from L1-L6 did not block the locomotor rhythm or significantly alter the phase of the left/right L1/2 ventral root discharge. When flexor activity could be suppressed, this resulted in transient changes in the phase of the left/right L1/2 ventral root activity and in some preparations the locomotor rhythm was transiently blocked. When the light was turned off, the amplitude of the locomotor bursts and sometimes their frequency was transiently increased for tens of seconds before returning to control levels. We conclude that flexor motoneurons provide feedback to the CPG during drug-induced locomotor-like activity that acts to stabilize the locomotor rhythm. Our findings are consistent with recent work

suggesting that flexor-related neurons are particularly important for genesis of the locomotor rhythm.

Disclosures: **M. Falgairolle:** None. **J.G. Puhl:** None. **A. Pujala:** None. **M.J. O'Donovan:** None.

Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant 1RO1NS091836-01

Title: Synaptic origin of synchronized bursting in the spinal cord and the role of gap junctions

Authors: ***A. MAHROUS**, S. ELBASIOUNY;
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Abstract: Rhythmic patterns of motoneuron activity in the spinal cord represent the basis for generating important motor behaviors such as locomotion and respiration. The cellular and network mechanisms responsible for generating these patterns are still not completely understood. We attempted to study the role of the small conductance calcium-activated potassium channels (SK channels) in generating synchronized bursts in the spinal cord. The synaptic conductances mediating the synchronized bursting were also investigated. In addition, a possible role of gap junctions in synchronizing the activity of motoneurons to generate this behavior was studied. We used the in-vitro sacral cord preparation from adult mice as it allows for different pharmacological manipulations. Synchronized bursting was induced pharmacologically and the motor output was measured both from the ventral roots as well as from single motoneurons. Our results show that SK channels play a major role in initiating and grading synchronized bursts in the spinal cord. We also found that the synaptic pathway involved in generating the synchronized bursting depends on both NMDA and AMPA receptors. Neither the burst characteristics nor the synchrony across different pools were affected by gap junction blockers. Consequently, a role of gap junction in mediating this synchronized bursting behavior is excluded. A common synaptic input to the motor pool is a more likely mechanism for synchrony.

Disclosures: **A. Mahrous:** None. **S. Elbasiouny:** None.

Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: Royal Society Research grant RG130560

Title: Functional recovery of a developing locomotor network following spinal cord injury

Authors: S. ANAGIANNI, *H. ZHANG;
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Abstract: Spinal cord injury in mammals including human is detrimental and can lead to complete loss of function, which is primarily due to the inability of neuronal axons to regenerate and reconnect to their appropriate targets beyond the injury site. In contrast, *Xenopus* tadpoles show tremendous regenerative capability and are able to swim again in a couple of days after spinal cord transection, providing an excellent model to investigate regeneration of spinal neural circuit following injury. Spinal neural circuits controlling rhythmic movements like swimming and walking are called central pattern generators (CPGs). It is still largely unknown how CPG networks recover following injury. Using the spinal cord of *Xenopus* tadpoles we aim to reveal a successful functional recovery at behavioral, network, and cellular levels. *Xenopus* embryos at the time of hatching (2 days old, stage 37/38) were used to perform complete spinal cord transection. After one-day recovery at stage 42, *Xenopus* larval swimming behavior was monitored using high speed video recording, which showed that *Xenopus* tadpoles regained the ability to freely swim, but the speed was lower. CPG network output (ventral root activity) was recorded simultaneously with single neuron whole-cell current-clamp recording in order to explore the recovery of rhythmic motor output following spinalisation and how the properties of injured CPG neurons change during functional recovery. Our results showed that rhythmic motor output was restored beyond the lesion site after complete spinal cord transection, but swimming episode duration was shorter and motor burst frequency was lower. Using neurobiotin-DAB staining, the extent of axonal regeneration of different classes of CPG neurons was also investigated. Understanding basic mechanisms of motor circuit repair after spinal cord injury can be beneficial for further studies on motor circuit regeneration in mammals and possible treatments for spinal cord injuries.

Disclosures: **S. Anagianni:** None. **H. Zhang:** None.

Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant R15HDO75207

Title: Properties of spinal locomotor circuitry during development in SMN deficient neonatal mice

Authors: *D. L. HIGGIN, J. LOMBARDO, M. HARRINGTON;
Dept. of Biol. Sci., Delaware State Univ., Dover, DE

Abstract: Rhythmic locomotor activity is present in the lumbar spinal cords of neonatal mice as early as the first post-natal day (P0) well before the emergence of walking behavior, but its functional role and the timeline for its functional development is unknown.
Recent work with mouse models of Spinal Muscle Atrophy (SMA) has shown that SMN-deficient motor neurons are impaired in central circuitry and synaptic function; however whether this loss effects rhythmic locomotor activity is unknown. Therefore, the neonates of SMA mouse models present an approach for determining the functional significance and developmental timeline of this early locomotor network maturation in a model where significant motor neuronal loss is expected.
Pharmacological application of Dopamine, NMDA, Serotonin, and Potassium has been shown to elicit locomotor-like activity and bath application in acutely isolated spinal cords from neonatal mice elucidate rhythmic alternating activity in the lumbar section of the spinal cord and contralateral ventral root discharge recordings of drug induced motor neuron activity are readily accomplished by suction electrodes.
We investigated spontaneous and drug induced rhythmic behavior in the lumbar spinal cord and compared control and SMA mice in early post-natal (P0 – P7) preparations, where rhythmicity has been shown to be established. We investigated rhythmicity in the network throughout various segmental levels of the lumbar spinal cord including those higher in the cord which are affected earlier in the course of the disease compared to those lower in the cord that are affected later.

Disclosures: D.L. Higgin: None. **J. Lombardo:** None. **M. Harrington:** None.

Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: DFG SM 206/3-1

UoC ZUK 81/1

Title: Descending input and its effect on a coordinating network

Authors: *F. CLOTTEN[1], C. R. SMARANDACHE-WELLMANN[2];
[1]Zoological Inst., Univ. of Cologne, Koeln, Germany; [2]Inst. of Zoology, Univ. of Cologne, Cologne, Germany

Abstract: The swimmeret system of the crayfish is an easily accessible model for studying locomotion. The segmental organization of the central nervous system provides the opportunity to elucidate the mechanisms of both, the intersegmental coordination of central pattern generators (CPGs) and the coordination of coupled CPGs within one segment (left-right coordination). The general network properties of the swimmeret system and the coordination of CPGs that underlies this coupled activity were previously investigated in detail. Consequently it is of great interest to understand the effect of descending input from the brain on the coordination of the swimmeret CPGs. Both excitatory and inhibitory command neurons of the swimmeret system were described but no information is available about the input these neurons receive or their neural targets within the swimmeret system.
In this comparative study separated axon bundles in the connectives of the abdominal nerve cord were stimulated electrically. Stimulation induced and terminated rhythmic activity in inactive and active preparations, respectively. Histological identification of the stimulation sides revealed that the locations of the stimulated axon bundles are consistent with previously described locations of excitatory and inhibitory command neurons. In the signal crayfish, *Pacifastacus leniusculus*, electrical stimulations affected both sides of the nervous system in the same manner. Rhythmic activity was initiated or terminated bilaterally to the same extent. In contrast, asymmetric rhythmic activity (i.e. rhythmic activity solely ipsilateral to the stimulated axon bundles) could be induced in the galician crayfish, *Astacus leptodactylus*. In intact crustaceans this behavior is known as a righting response of the swimmeret system due to spatial movements of the animal. Bath application of carbachol, a nicotinic and muscarinic analog of acetylcholine that increases the excitation of the swimmeret system, increased the stimulation effect on the side of the abdominal nerve cord that was contralateral to the stimulation side. This suggests that the ipsilateral or bilateral initiation of swimmeret movements depends on the system's excitation level.

With increasing stimulation frequencies the period of the evoked rhythmic activity decreased and more motor neurons were recruited. These results, in addition with intracellular recordings of motor neurons during sub-threshold stimulations, give evidence that both the swimmeret motor neurons and presynaptic interneurons of the pattern-generating micro-circuits are possible targets of the command neurons within the swimmeret system.

Disclosures: F. Clotten: None. **C.R. Smarandache-Wellmann:** None.

Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant 5T32GM008471-23

NSF Grant 1454904

Title: Ultrasound-induced inhibition of neural activity in an invertebrate motoneuron

Authors: *M. NEWHOFF[1], W. LEGON[2], K. A. MESCE[1];
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Abstract: Ultrasound (US) is a promising noninvasive neuromodulation technology with the potential to confer the high spatial resolution of invasive techniques used to treat a wide range of neurological disorders, including epilepsy and Parkinson's disease. Although researchers have known for several decades that ultrasound is capable of altering neural firing rates, the underlying cellular mechanisms accounting for such changes have remained elusive. Several hypotheses regarding its mode of efficacy include formation of microbubbles (cavitation), generation of heat, and mechanical distortion. To gain a greater understanding of the cellular mechanisms underlying the actions of US, we utilized simultaneous ultrasonic stimulation and single-unit electrophysiological recordings in a well-studied invertebrate model, the medicinal leech *Hirudo verbana*. This preparation provides an especially accessible physiological system in which identified neurons can be studied at the level of their membrane biophysical properties, circuit connections and behavioral roles. One well-studied motoneuron involved in locomotion, the Dorsal Longitudinal Muscle Excitor (DE-3), has one of the largest axons in its nerve root, and thus can be unambiguously detected via extracellular recording. Using ultrasound at a frequency of 960 kHz, we were able to inhibit repeatedly and reversibly the firing rate of DE-3

(N=6), and by 50-100% (N=4). The robust restoration of firing rates argues against the idea that cavitation and its deleterious effects on cells were causal to the US-induced changes observed. Intriguingly, US also rapidly switched tonic DE-3 firing over to a bursting mode in the presence of dopamine, a phenomenon that may have resulted as a consequence of hyperpolarization-activated depolarizing inward currents. Repeated application of US did result in a maximum temperature increase of 2°C, but such fluctuations alone did not affect neuronal firing rates appreciably. We conclude that the effects of US on DE-3 likely involve activation of ion channels mediating hyperpolarizing conductance states. Furthermore, because the neuron that we tested with US was a motoneuron, as opposed to a mechanosensory neuron, we have the opportunity to understand better how US alters a host of ion-channels that are not biologically maximized for mechanical gating (e.g. TRP channels). Pharmacological manipulations in the presence of US are currently being carried out to determine the identity of ion channels affected by US stimulation. By gaining a greater understanding of the mechanisms by which US enacts changes in neural activity, we hope to increase its therapeutic potential.

Disclosures: **M. Newhoff:** None. **W. Legon:** None. **K.A. Mesce:** None.

Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

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Support: DFG SM206/3-1

Title: Synapses between encoders and decoders: understanding a coordinating network

Authors: ***F. BLUMENTHAL**[1], C. R. SMARANDACHE-WELLMANN[2];
[1]Zoological Institute, Animal Physiology, Emmy-Noether Group, Univ. of Cologne, Koeln, Germany; [2]Zoological Institute, Animal Physiology, Emmy-Noether Group, Univ. of Cologne, Cologne, Germany

Abstract: The swimmeret system is an excellent model to understand neural mechanisms of coordination of distributed central pattern generators (CPG). During swimming the four paired swimmerets of the crayfish's abdomen are coordinated in an anteriorly proceeding metachronal wave. Each swimmeret is innervated by neurons from its own module, containing a CPG, these have to be coordinated among each other. The intersegmental coordination of four ipsilateral, anatomically separated CPGs is achieved by three neurons located in each hemisegment forming a coordinating circuit. One ascending (ASC_E) and one descending (DSC) coordinating neuron

encode the information about the status of their home module and project it to the other neighbors. A nonspiking neuron, Commissural Interneuron 1 (ComInt1), decodes this information transmitted by three neighboring coordinating neurons and integrates it into its own CPG. ComInt1 receives these inputs with a gradient of synaptic strength, where the strongest excitatory postsynaptic potential (EPSP) is always elicited by the ASC_E from the posterior neighbor. The anterior DSC elicits a weaker EPSP and the most distant coordinating neuron has the weakest input.
Here we want to investigate if the gradient of synaptic strength is due to the morphology of synaptic contacts. For these experiments, ComInt1 and coordinating neurons were filled with fluorescent dye and the presynaptic boutons of the coordinating neurons on ComInt1 were marked immunohistochemically with Anti-Synapsin. An immunohistochemical labeling of the postsynapses of ComInt1 with PSD-95 was so far not successful.
The axons of the coordinating neurons run dorsally, parallel to the midline through each segment. ComInt1 has its soma in one hemisegment, sends its primary neurite dorsally over the midline to the lateral neuropil on the contralateral side where it forms an electrical synapse with one of the CPG neurons. ComInt1 has one ascending and one descending dendritic branch parallel to the midline and to the axons of the coordinating neurons.
At the midline we identified synapses of the coordinating neurons by colocalized presynaptic boutons with an intracellular stained axon. The colocalizations were dorsally all along and strictly parallel to the midline of the ganglion. These colocalizations do not yet explain the three distinct sizes of EPSPs in ComInt1 but it is a first approach to investigate this morphologically. We will perform more double stainings of two different coordinating neurons and presynaptic boutons as well as triple stainings with ComInt1 to investigate if the areas of colocalization and the number of synapses vary in accordance to the synaptic inputs.

Disclosures: F. Blumenthal: None. **C.R. Smarandache-Wellmann:** None.

Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: DFG, RTG 1960

Title: Ionic currents and their influence on rhythm generation and coordination

Authors: *L. SCHLAEGER[1], C. R. SMARANDACHE-WELLMANN[2];
[1]Dept. of Animal Physiol., [2]Inst. of Zoology, Animal Physiology, Emmy-Noether Group, Univ. of Cologne, Cologne, Germany

Abstract: The function of nervous systems is based on the interaction of neuronal networks. These networks are built of neurons with individual activity patterns that together regulate and coordinate complex movements and behavior. To better understand the properties of such networks, we investigate the crayfish swimmeret system. Swimmerets are four pairs of abdominal limbs that move in alternating power- and return strokes in a metachronal wave from posterior to anterior. In each hemiganglion a similar subset of neurons can be found that drives this movement. Each microcircuit is composed of five interneurons forming the rhythm generating circuit, three coordinating neurons, and 70 motor neurons (MN). When the system is active, all of these neurons show membrane potential oscillations but with distinct activity patterns. Despite our good understanding of the cellular components and synaptic contacts among them, the intrinsic mechanisms that enable the individual activity pattern still remain unknown. Therefore we are interested in the ionic currents underlying the similar yet different activity patterns of the neurons and how they influence rhythm generation and coordination between segments.
We performed single electrode current- and voltage-clamp recordings from dendritic aborizations in the isolated abdominal nervous system of the crayfish, *Pacifastacus leniusculus*. To identify and reveal different ionic currents we bath applied selective ion-channel blockers. In this system we did not find any indication of the existence of the hyperpolarizing activated cation current (I_H). This is remarkable since it was shown in other systems to have key importance for generating membrane potential oscillations. This led to further investigations regarding the origin of the neuronal activities. After application of channel blockers against the high voltage activated calcium current I_L (Nifedipine), the transient potassium current I_A (4-AP) or the persistent sodium current I_{NaP} (Riluzole) we could observe an altered ability of the system to produce a steady and coordinated motor rhythm. This led to the conclusion, that the activities of the pattern generating, as well as the coordinating neurons, are dependent on these currents. When analyzing the effect of the channel blockers on input resistances of MNs, Nifedipine had the strongest effect in increasing the resistances. The other blockers did not change the input resistances significantly. However, the amplitude of membrane potential oscillations declined under application of all channel blockers. Resulting, we anticipate a major importance of I_L and a minor relevance of I_{NaP} and I_A for motorneuronal activities.

Disclosures: L. Schlaeger: None. **C.R. Smarandache-Wellmann:** None.

Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

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UoC ZUK 81/1

Title: Differential tuning of neurons in a coordinating circuit

Authors: *A. C. SCHNEIDER[1], F. BLUMENTHAL[2], C. R. SMARANDACHE-WELLMANN[2];
[1]Univ. of Cologne, Koeln, Germany; [2]Inst. of Zoology, Animal Physiology, Emmy-Noether Group, Univ. of Cologne, Cologne, Germany

Abstract: Neural oscillators coordinated to produce meaningful behavior exist in a large variety of animals, ranging from simple locomotor networks to brain oscillations. A model system to study coordination of distributed neural oscillators is the crayfish swimmeret system. Its neurons, especially those for coordination, are identified and their connections characterized.
These neurons drive four pairs of swimmerets in a metachronal wave with 25% phase lag between segments. Each swimmeret is controlled by its own central pattern generator (CPG) located in the corresponding abdominal hemisegment. Necessary and sufficient to coordinate CPGs across segments are three neurons per hemisegment: The ascending (ASC_E) and descending (DSC) coordinating neuron, and the non-spiking Commissural Interneuron 1 (ComInt 1). ASC_E and DSC, driven by the CPG, encode information about their home ganglion's activity state and send it as corollary discharge to the ComInt 1s of the other ganglia. Each action potential of ASC_E and DSC causes a distinct and fast excitatory postsynaptic potential in the target ComInt 1, which integrates the coordinating information in the target CPG.
The 25% phase lag is independent of frequency and strength of swimmeret movements. Therefore, we hypothesized that changes in the system's excitation tune the encoding and decoding properties of the coordinating circuit. We used isolated abdominal nerve cords to record intracellularly from ASC_E, DSC or ComInt 1. Excitation was changed by bath application of different carbachol (cholinergic agonist) or edrophonium chloride (acetylcholine esterase inhibitor) concentrations.
To investigate direct and indirect actions of the drugs we measured input resistance (Rin) and membrane potential of these neurons both in the intact network and chemically isolated. Rin of ASC_E decreased, of DSC increased, and of ComInt 1 did not change with increasing drug concentration. Only when isolated from the network with TTX in low Ca^{2+}/high Mg^{2+} saline drug concentration had the opposite effect on ASC_E's and DSC's Rin, and their membrane

potential depolarized. These opposing effects show that the direct effects of system excitation were usually masked by indirect network effects. ComInt 1 was largely unaffected by changes in excitation. We conclude that the differential direct and indirect effects on the coordinating neurons' tune their encoding properties. As ComInt 1 is not subject to excitation it cannot entirely decode coordinating information but may rather work as hub neuron. Decoding with respect to excitation may then take place in the CPG itself, which is also one source of tuning for the coordinating neurons.

Disclosures: A.C. Schneider: None. **F. Blumenthal:** None. **C.R. Smarandache-Wellmann:** None.

Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: CFI #33817

NSRIT #33817

NSERC R6P1N-2015-0871

Title: Crossed reflex pathways in freely walking mice

Authors: *O. D. LAFLAMME[1,2], T. AKAY[1,2];
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Abstract: The commissural neuronal circuitry, involved in left-right coordination, has been investigated at a cellular and functional level by combining *in vivo* experiments in cats and *in vitro* electrophysiological experiments with mouse genetics. These experiments identified several genetically distinctive commissural pathways and their possible role in transmitting the activity of central pattern generators (CPG) underlying locomotion from one side of the body to the contralateral side. Investigations with the cat unraveled several crossed reflex pathways, but the role of these pathways during walking and their relation to the commissural pathways identified in mice remained unknown. The overall aim of our study is to characterize crossed reflex pathways in mice that would enable connections to be formed between the crossed reflex data from cat experiments and the more recent commissural CPG pathways from mouse experiments. Here, we describe crossed reflex pathways in mice induced either by proprioceptive or cutaneous afferent signals. We chronically implanted nerve stimulation electrodes to activate cutaneous

(Sural nerve) and proprioceptive feedback from flexor (Peroneal nerve) and extensor (Tibial nerve) muscles in fully awake mice during resting and walking on a treadmill. In parallel, electromyogram activities from multiple flexor and extensor muscles of the contralateral leg were recorded to document reflex responses transmitted to the contralateral side of the spinal cord. Electrical stimulation of all three nerves evoked motor responses on the contralateral side of the body in every muscle investigated. All muscles were activated approximately at the same latency, except for the tibialis anterior, which was slightly faster. Moreover, the temporal aspect of this pattern remained similar during resting and walking. The amplitude of flexor and extensor muscle activation in response to sural nerve stimulation was consistently stronger during walking than during resting. However, during proprioceptive nerve stimulation, regardless of whether from flexor or extensor muscle, the extensor muscle response decreased, while flexor activity either slightly increased or remained the same during walking compared to resting. We describe the crossed reflex in mice and show that the amplitude of the flexor and extensor muscle activities, but not the temporal structure of muscle response is modulated during walking compared to resting.

Disclosures: O. D. Laflamme: None. **T. Akay:** None.

Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 626.17/XX18

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH NS083319 (DB & FN)

Title: The roles of different potassium currents for action potential propagation

Authors: *N. DAUR, F. NADIM, D. BUCHER;
Dept. of Biol. Sci., NJIT and Rutgers Univ., Newark, NJ

Abstract: Most neurons express multiple types of voltage-gated ion channels in their axons, giving rise to nonlinear membrane behavior beyond simple spike propagation with just fast Na^+ and delayed rectifier K^+ currents. One consequence is spike history-dependent change in conduction velocity that goes far beyond simple refractory effects and can substantially alter the interval structure of repetitive activity. This implies that axonal propagation can potentially influence the temporal code of neural communication.
We have previously described changes in conduction velocity in rhythmically active neurons in the lobster stomatogastric nervous system (Ballo et al., J Neurosci 2012). Spike delay along the

4-5 cm axon of the pyloric dilator (PD) neuron depends on mean activity rate, as repeated bursting hyperpolarizes the axon and increases delay with a time constant of several minutes. This slow time scale increase in delay is accompanied by an increase in delay variability: after several minutes of activity, delay can change by ~30% within a single burst. The slow time scale hyperpolarization depends on activation of a Na^+/K^+ pump, which is balanced by a hyperpolarization-activated current (I_h).
A computer model of the axon shows that the fast time scale variability is due to the effect of changing resting potential on the gating variables of the Na^+ current. Because Na^+ current gating depends on the activation levels of K^+ currents, we explore the contribution of K^+ currents to temporal fidelity. Voltage-clamp recordings of the PD axon reveal delayed rectifier (I_{Kd}) and A-type (I_A) currents, but no calcium-dependent K^+ currents. Complete block of I_{Kd} with TEA results in repetitive firing in response to a single nerve stimulus. In contrast, block of I_A with 4-AP substantially increases spike duration, but does not lead to repetitive firing. I_{Kd} and I_A also have differential effects on temporal fidelity. Partial block of I_{Kd} with low concentrations of TEA has little effect on the variability of conduction delay at the beginning of repeated burst stimulation, but substantially alters it after several minutes of stimulation, when the resting membrane potential is more hyperpolarized. In contrast, partial block of I_A with low concentrations of 4-AP substantially alters variability of delay at the beginning of stimulation, but has little effect after several minutes, ensuing activity-dependent hyperpolarization. Therefore, the contribution of these different K^+ currents differentially depends on the history of spiking activity itself, and may affect different aspects of axonal function. We explore the underlying ionic conductance mechanisms using our axon model.

Disclosures: **N. Daur:** None. **F. Nadim:** None. **D. Bucher:** None.

Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 626.18/XX19

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant MH051393

NIH Grant NS066587

NIH Grant NS070583

Title: A coactivated command like neuron modifies the dynamics of motor program articulation.

Authors: *C. G. EVANS[1], K. R. WEISS[2], E. C. CROPPER[2];
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Abstract: In many invertebrate model systems, it has been shown that multiple command-like neurons are activated to initiate or maintain a specific behavior. In principle this may suggest there is redundancy, or that each command-like neuron has a unique function, contributing to specific parameters of the behavior. Although data in a number of contexts are beginning to favor the latter view, little is known about these individual functions. We address this issue in the well described feeding system of *Aplysia californica*. In *Aplysia*, motor output from the buccal ganglia can drive egestive and ingestive behaviors. A population of about 13 cerebral-buccal interneurons (CBIs) project to the buccal ganglia and are higher-order interneurons that can activate or modulate feeding motor programs. The most extensively characterized is CBI-2. It is activated by chemical (food) and tactile stimuli to the lips and tentacles and strongly and reliably drives motor programs in the buccal ganglia. It will initiate feeding movements in semi intact preparations. When CBI-2 is made to fire repeatedly in bursts at fixed intervals the motor programs gradually convert from initially intermediate to ingestive. We have termed this effect, repetition priming. CBI-3 is also activated by food, and has been found to fire with CBI-2 during protraction in feeding intact-head preparations. When driven to fire with CBI-2, in isolated ganglia, CBI-3 immediately converts an intermediate or egestive program into an ingestive program. In the present study we examine the effect of firing CBI-3 at a physiologically relevant frequency simultaneously with CBI-2 during repetition priming. We show that co-activation of CBI2 and CBI3 results in a faster transition from intermediate to ingestive motor programs than with stimulation of CBI-2 alone. Therefore one of the functions of a co-activated command-like neuron during feeding behavior is to modify the dynamics of motor programs induced by the other.

Disclosures: C.G. Evans: None. **K.R. Weiss:** None. **E.C. Cropper:** None.

Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 626.19/XX20

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant R01-NS26539

NIH Fellowship F32-NS083099

Title: The spatial topography of cranial motor neuron activity patterns in larval zebrafish revealed by confocal and whole brain light-sheet calcium imaging

Authors: *K. L. MCARTHUR, J. R. FETCHO;
Neurobio. & Behavior, Cornell Univ., Ithaca, NY

Abstract: A neuron's location in the developing neuroepithelium both reflects its ontogenetic history and predicts its functional future. Spatial signaling gradients drive differential gene expression, such that specific neuronal subtypes reliably arise in particular brain locations. Spatial location is further refined by neuronal migrations - including some dramatic repositioning of entire populations and more subtle relative positioning within each population. Ultimately, the location of a neuron's cell body and extensions will determine its access to presynaptic and neuromodulatory inputs - though the relationship between location and connectivity may be more or less flexible for specific synaptic partners. To understand the relationship between location and function in the developing hindbrain, we focus on the facial branchiomotor neurons (FBMNs) in larval zebrafish, a group of cranial motor neurons driving respiration and feeding. These neurons adopt a clear age topography early in development and serve as a general model for the relationship between dorsoventral location, age, and function in cranial motor pools. Further, because these neurons execute an early and dramatic caudal migration in wild type animals (but not in specific mutant lines), we have the opportunity to study the impact of abnormal population positioning on circuit architecture throughout the hindbrain. We mapped FBMN activity patterns in three-dimensional space, using confocal and light sheet imaging of genetically encoding calcium indicators. We found a loose functional topography, where FBMNs exhibiting higher-frequency respiratory bursts (in addition to swim-related activity) are concentrated in the ventrolateral portion of the motor nuclei in wild type larvae. These results are consistent with previous backfill data regarding the location of motor pools known to participate in respiratory behaviors. We also probed for neurons that have correlated activity with FBMNs. This revealed neurons with potential functional relationships to the FBMNs, including other motor neurons and potential presynaptic interneurons. This wild type data provides a basis for comparison to whole-brain activity in mutants, to discover to what extent the spatial structure of hindbrain neuronal activity is altered when a critical motor nucleus is abnormally positioned. Leveraging whole-brain calcium imaging in this way, in this specific group of neurons - when combined with single-cell morphology and electrophysiology - will finally allow us to test hypotheses about the degree to which large-scale circuit architecture depends on proper positioning of neuronal populations.

Disclosures: K.L. McArthur: None. **J.R. Fetcho:** None.

Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 626.20/DP08 (Dynamic Poster)

Topic: E.07. Rhythmic Motor Pattern Generation

Support: SNF APM

Title: Imaging neural dynamics governing *Drosophila* limb control during walking and grooming

Authors: *P. RAMDYA;
Caltech, Pasadena, CA

Abstract: Motor control circuits cannot be fully understood without measuring their dynamic activity during behavior. Until now, it has been challenging to image the activity of vertebrate and invertebrate limb coordination circuits due to their inaccessibility. Here, I introduce a preparation that permits 2-photon calcium imaging of neural activity in the ventral nerve cord of tethered, behaving *Drosophila melanogaster*. A dorsal thoracic dissection uncovers neurons that control prothoracic and mesothoracic legs as well as populations of descending and ascending interneurons that pass through the cervical connective. I will demonstrate how this preparation can reveal the dynamics of peripheral sensory neurons, interneurons for inter-leg coordination, neuromodulatory neurons, and motor neurons while animals walk and groom on a spherical treadmill. This approach provides an indispensable tool for uncovering how a relatively compact motor control system can orchestrate complex behaviors.

Disclosures: P. Ramdya: None.

Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 626.21/XX21

Topic: E.07. Rhythmic Motor Pattern Generation

Support: CIHR MOP 86470

CFI Project# 30852

Title: Anatomical and electrophysiological characterization of WT1-expressing neurons in the mammalian spinal cord.

Authors: F. HAQUE[1], W. ZHANG[2], *S. GOSGNACH[3];
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Abstract: The neural network underlying locomotion (i.e. locomotor CPG) in mammals is comprised of interneurons located in the ventromedial aspect of the lumbar spinal cord, and is able to generate rhythmic locomotor-like motor patterns in the absence of sensory input or descending inputs from the brain. Traditional methods of investigating the structure and mechanism of function of this neural circuit have involved identifying and characterizing single component interneurons based on electrophysiological and anatomical criteria. This approach has proven to be extremely low yield due to the large number of interneurons present in the mammalian spinal cord combined with the fact that cells with a similar function are intermingled with other, functionally unrelated, interneurons. Our lab implements a molecular genetic approach to label entire interneuronal populations with a similar genetic lineage and study each genetically-distinct population in order to characterize their anatomical and electrophysiological properties, as well as their potential role in generating locomotor outputs. Recently we have focused on the dI6 population, a group of interneurons that originate in the dorsal region of the developing neural tube, migrate ventromedially, and settle in lamina VIII of the functionally mature spinal cord. dI6 cells can be divided up into at least 3 subsets based on transcription factor expression: WT1+ cells; DMRT3+ cells; and WT1+/DMRT3+ cells. Previous work demonstrated that the DMRT3+ population is primarily inhibitory and have axons that contact both ipsilateral and contralateral motor neurons. Our work investigates the detailed morphology, neurotransmitter phenotype, and electrophysiological properties of the WT1+ and WT1+/DMRT3+ cells. Results indicate that both subsets are primarily GABAergic, and that the axonal projection pattern of WT1+ cells is distinct from that of the DMRT3+ subset. Furthermore, the vast majority of the WT1+ cells are rhythmically active during locomotion suggesting that these cells may be a component of the locomotor CPG.

Disclosures: F. Haque: None. **W. Zhang:** None. **S. Gosgnach:** None.

Poster

626. Central Pattern Generating Circuits: Motor Neurons and Interneurons

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 626.22/XX22

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NS080586

NS086372

Embo Long-Term Fellowship

Title: Partially shared inhibitory and excitatory spinal circuits for withdrawal reflex, scratch and locomotion

Authors: *G. GATTO, M. GOULDING;
Salk Inst. For Biol. Studies, LA Jolla, CA

Abstract: Animals display a highly varied repertoire of motor behaviors that reflect the patterned activity of a limited number of muscles and motor neurons. Rhythmic behaviors, such as walking or breathing, are generated by the activity of neuronal networks, known as central pattern generators (CPGs). Work in turtles has shown a partial overlap of the CPG neurons that are active during locomotion and scratching. To date, these neurons have not been molecularly identified. Using a tripartite intersectional genetic approach, we have investigated the role of specific interneuron populations during reflex responses and locomotion. Spinal ablation of V1 (En1-Cre) and V2a (Chx10-Cre), but not V2b (Gata3-Cre) interneurons in adult mice leads to impaired responses to chloroquine-induced scratch. Among the analyzed mice, ablation of V1 or V2a interneurons causes a strong reduction in the speed of scratching, and in the number of scratch bouts, suggesting that these interneuron populations have an essential role in enabling fast scratch movements. Acute silencing of these neurons, using CNO-activated hM4D receptor, recapitulates the slower velocity during scratching. Interestingly, ablation of V1 or V2a interneurons has already been shown to impair mice performance at high locomotory speeds, implying that scratch and locomotion share the CPG neurons responsible for facilitating fast movements. We also find an impaired response to mechanical stimuli but not noxious-induced paw withdrawal in V1 ablated mice, suggesting that the withdrawal reflex may recruit different interneuron pathways according to the applied stimulus.
Taken together, these data identify two key CPG populations as common elements in the CPG circuits for rhythmic scratch and locomotion. Our data also infer that V2a interneurons might be part of the scratch-rhythm generator, while the V1 interneurons are required for the fine-tuning of the speed. In the future, it will be important to address how these two populations are connected and interact during scratch (a unilateral rhythmic behavior) and locomotion (a bilateral coordination of rhythm).

Disclosures: **G. Gatto:** None. **M. Goulding:** None.

Poster

627. Spinal Cord Injury: Rehabilitation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 627.01/YY1

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH Grant R15NS082711

Title: The effectiveness of treadmill training is enhanced by applying robotic resistance in a rodent model of spinal cord injury

Authors: *C. A. ESTRADA[1], E. J. HINAHON[2], E. RUIZ[3], T. TRAUGHBER, Jr[2], S. VILLANUEVA[1], S. SIROT[1], M. ROBLES[1], R. MARTINEZ[1], D. WON[4], R. DE LEON[2]; [1]Dept. of Biol. Sci., [2]Sch. of Kinesiology and Nutritional Sci., [3]Dept. of Chem. and Biochem., [4]Col. of Engineering, Computer Science, and Technol., Cal State LA, Los Angeles, CA

Abstract: There is evidence that robotic-assisted treadmill training with body weight support did not improve the recovery of stepping patterns after a spinal cord injury (SCI). Applying robotic resistance, however, may be beneficial although, to date, proper controls have not been included in experiments of robotic resistance. We examined the effects of robotic-applied resistive forces in a rodent model of SCI. Forty female Sprague Dawley rats received a severe mid-thoracic spinal cord contusion injury. A baseline test was performed two weeks later, after which treadmill training began. A robotic device was used to apply viscous forces that opposed horizontal or vertical movements of the hindlimbs during treadmill training. One group rats (n=10) was trained with a robotic horizontal force (HF) and another group (n=10) was trained with a vertical force (VF). Control groups consisted of a group of rats receiving treadmill training without robotic resistance (n=10) and a group of untrained rats (n=10). Training was performed for 15 minutes/day, 5 days/week. After 6 weeks of training with robotic resistive forces, improvements in step cycle kinematics were observed based on ankle position data collected by the robotic device. Specifically, training with the HF significantly increased step length and forward displacement by 24% and 16%. Training with VF did not affect step length parameters but significantly improved step height by 26%. Training with VF also significantly increased peak vertical velocity during lift and peak vertical velocity prior to paw contact by 49% and 40% respectively. The percentage of weight bearing steps only improved in the rats trained with VF. No significant changes were observed in untrained controls or in rats that were trained without the resistive forces. These findings suggested for the first time that adding resistive forces enhanced the effectiveness of weight supported treadmill training. HF and VF shaped the step cycle in a direction-dependent manner, but training with the vertical resistance (which has not yet been studied) may be more beneficial than horizontal resistance. These

findings have implications for the use of robotic technology in treadmill training therapies following SCI.

Disclosures: **C.A. Estrada:** None. **E.J. Hinahon:** None. **E. Ruiz:** None. **T. Traughber:** None. **S. Villanueva:** None. **S. Sirot:** None. **M. Robles:** None. **R. Martinez:** None. **D. Won:** None. **R. de Leon:** None.

Poster

627. Spinal Cord Injury: Rehabilitation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 627.02/YY2

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH Grant R15NS082711

Title: treadmill training with robotic resistance enhances synaptic plasticity in spinal cord injured rats

Authors: *E. RUIZ[1], S. VILLANUEVA[2], C. ESTRADA[2], E. HINAHON[3], T. TRAUGHBER, Jr.[3], S. SIROT[2], M. ROBLES[2], R. MARTINEZ[2], D. WON[4], R. DE LEON[3];
[1]Dept. of Chem. and Biochem., [2]Dept. of Biol. Sci., [3]Sch. of Kinesiology and Nutritional Sci., [4]Col. of Engeneering, Computer Sci. and Technol., Cal State LA, Los Angeles, CA

Abstract: There is evidence that applying resistive forces during treadmill training improves the recovery of stepping patterns after a spinal cord injury (SCI). Whether the effects of treadmill training are augmented by the addition of resistive forces augments is not known. The question we asked here was whether adding resistive forces enhanced plasticity in the rat spinal cord more so than treadmill training alone. Forty female Sprague Dawley rats received a severe mid-thoracic spinal cord contusion injury. A baseline test was performed two weeks later, after which treadmill training using a robotic device to apply viscous forces that opposed horizontal or vertical movements of the hind limbs. One group of rats (n=10) was trained with a robotic horizontal force (HF), another group (n=10) was trained with a vertical force (VF), another group was trained (T) without force (n=10) and one group was untrained (UT) (n=10). Training was performed for 15 minutes/day, 5 days/week. After 6 weeks of training, the rats were killed and the spinal cords removed and processed for immunohistochemistry. We performed experiments to quantify biochemical markers for synaptic plasticity, i.e. synatophysin, VGLUT1 and GLYT2, in the ventral horn of the lumbar spinal cord. The expression of synaptophysin was significantly greater after HF training compared to VF and T training ($p<0.05$). No significant differences were found for VGLUT1 and GLYT2 expression in the ventral horn. These preliminary data

suggested that HF training increased the amount of synapses in the lumbar spinal cord more so than other forms of training. The enhanced synaptic plasticity may be associated with the observed beneficial effects of training with horizontal resistance. On-going experiments with VGLUT and GLYT2 expression around labeled motor neurons will provide greater insight into the nature of these synapses. These findings have implications for the use of treadmill exercise therapies following SCI.

Disclosures: **E. Ruiz:** None. **S. Villanueva:** None. **C. Estrada:** None. **E. Hinahon:** None. **T. Traughber:** None. **S. Sirot:** None. **M. Robles:** None. **R. Martinez:** None. **D. Won:** None. **R. de Leon:** None.

Poster

627. Spinal Cord Injury: Rehabilitation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 627.03/YY3

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH Grant R15NS082711

Title: The effects of weight supported quadrupedal treadmill training in a rat model of spinal cord injury and metabolic syndrome

Authors: *T. TRAUGHBER, JR[1], E. RUIZ[2], C. ESTRADA[3], E. ARCENA[1], S. VILLANUEVA[3], S. DERIQUITO[1], R. DE LEON[1];
[1]Sch. of Kinesiology and Nutritional Sci., [2]Dept. of Chem. and Biochem., [3]Dept. of Biol. Sci., Cal State LA, Los Angeles, CA

Abstract: Weight supported treadmill training improves walking in individuals with spinal cord injured (SCI). Because it is a form of exercise, it may also reduce the risk factors associated with metabolic syndrome which is prevalent in the SCI population. In this study, we examined the effects of imposing quadrupedal body weight supported treadmill exercise in a rodent model of SCI and metabolic syndrome. Seventeen rats received a severe spinal cord contusion (T10). One day after the contusion, the rats were placed on a high energy diet consisting of 10% fat, 20% sucrose, and 70% normal rodent chow and 40 days later, a low dose of streptozotocin (30 mg/kg, i.p.) was administered. The rats were assigned to a trained (n=8) or non-trained group (n=9). Training consisted of weight supported, quadrupedal treadmill walking (23 cm/s) for 1 hr/day, 5 days/week for 6 weeks. Insulin tolerance tests were performed, hemodynamic measurements were recorded, and heart and hindlimb muscle tissue was harvested after the rats were killed. Based on fasting blood glucose, the high energy diet and STZ treatment was successful in

inducing hyperglycemia (i.e. >140 mg/dL; trained: 202±50 mg/dL; non-trained: 146±29 mg/dL). Fasting blood glucose levels were not significantly different between the groups. However, insulin sensitivity was improved by weight supported, quadrupedal treadmill training based on insulin tolerance test. We found significant differences in glucose levels 45 and 60 min after insulin injection indicating a greater insulin sensitivity in the trained group. No difference in insulin sensitivity was found at baseline. Training also significantly increased tibialis anterior, gastrocnemius and soleus muscle weights by 7%, 20%, 31% respectively indicating the training was effective in activating hindlimb activity in the SCI rats. No significant differences in body mass, heart weight or hemodynamic measurements were found between the groups. These findings suggest that weight supported treadmill exercise training can reduce some metabolic abnormalities that occur after SCI. The mechanism is unclear, but might involve adaptations in leg muscles which result in improved glucose utilization.

Disclosures: **T. Traughber:** None. **E. Ruiz:** None. **C. Estrada:** None. **E. Arcena:** None. **S. Villanueva:** None. **S. Deriquito:** None. **R. de Leon:** None.

Poster

627. Spinal Cord Injury: Rehabilitation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 627.04/YY4

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH Grant R15NS082711

Title: A new, simple device for facilitating weight supported overground and treadmill locomotion in spinal cord injured rats

Authors: *S. G. DERIQUITO[1], S. SIROT[2], C. GONZALEZ[1], T. TRAUGHBER, Jr.[1], D. REINKENSMEYER[3], R. DE LEON[1];
[1]Sch. of Kinesiology and Nutritional Sci., [2]Dept. of Biol. Sci., Cal State LA, Los Angeles, CA; [3]Dept. of Mechanical and Aerospace Engin., Univ. of California Irvine, Irvine, CA

Abstract: Previous studies of weight supported training use robotic devices that place the rodents in unnatural bipedal walking conditions. We developed the BART device (Body weight supported, ambulatory, rodent trainer) to train rodents in their more natural, quadrupedal gait (Hamlin et al., J Neurosci Methods.246:134-41, 2015). However, with the BART device only a limited number of steps was possible. We developed circular BART or CBART to exercise both the hindlimbs and forelimbs during weight supported overground and treadmill locomotion. The CBART device consists of a lever arm that hinges on a fulcrum. One advantage of the simple

design is that CBART can be easily constructed using parts obtained from any hardware store. The rat is placed at one end of the arm and is lifted by a weight placed on the other end of the arm. The amount of weight support is controlled by adding more weight or moving the location of the weight on the lever arm. The rats walk along a circular path during overground locomotion because the arm rotates around a vertical axis. We performed preliminary tests of CBART in Sprague Dawley rats that received a severe mid-thoracic spinal cord contusion injury (SCI (n=4) and in normal rats (n=4). Ink was placed on the skin above bony landmarks for the ileum, hip, ankle and metatarsal-phalangeal joint and the limbs were recorded by a small camera placed on the CBART arm. The recorded sequences were analyzed on a computer using MATLAB software that was developed in-house. After two weeks of training, all the rats learned to perform the overground locomotor task while attached to CBART with and without weight support provided. The effect of adding weight support was to decrease step length by 21% and increase hip-ankle-toe angle by 10%, consistent with our previous studies. During quadrupedal treadmill locomotion, CBART was effective in quantifying differences between poor and good stepping spinally contused rats. For example, hindlimb step length was significantly greater in good steppers compared to poor steppers (4.25±1.2 vs 0.56±0.5 mm). Ongoing analyses is being performed that will provide more details of kinematic characteristics. These preliminary data suggested that the CBART device facilitated quadrupedal overground and treadmill locomotion. Moreover, CBART is simple, inexpensive and easy to construct. These results suggested that the CBART device will be a useful tool for studying more natural forms of locomotion in SCI rats.

Disclosures: S.G. Deriquito: None. **S. Sirot:** None. **C. Gonzalez:** None. **T. Traughber:** None. **D. Reinkensmeyer:** None. **R. de Leon:** None.

Poster

627. Spinal Cord Injury: Rehabilitation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 627.05/YY5

Topic: C.09. Brain Injury and Trauma

Support: ANR-KCC2

SATT

Title: New therapeutic strategy to treat spasticity by targetting KCC2 after spinal cord injury

Authors: S. LIABEUF[1], L. STUHL GOURMAND[1], P. BOULENGUEZ[1], F. GACKIERE[1], A. VIALLAT LIEUTAUD[1], R. MANCUSO[1], L. VINAY[1], *F. BROCARD[2];
[1]CNRS, AMU, Marseille, France; [2]CNRS, Marseille, France

Abstract: In healthy adults, activation of γ-aminobutyric acidA (GABAA) and glycine receptors inhibits neurons as a result of low intracellular chloride concentration, which is maintained by the potassium-chloride cotransporter KCC2. A reduction of KCC2 expression or function is involved in the pathogenesis of several neurological disorders including spasticity following spinal cord injury (SCI). Given the critical role of KCC2 in regulating the strength and robustness of inhibition, identifying tools that may increase KCC2 function and hence restore endogenous inhibition in pathological conditions is of particular importance. The screening of a library of marketed drugs enabled to identify a family of compounds that are able to activate KCC2 function and reduce spasticity after spinal cord injury. We showed that the prochlorperazine, a member of this family, tested in vitro on the isolated neonatal rat spinal cord, was able to hyperpolarize the chloride equilibrium potential in motoneurons and strengthen the reciprocal inhibition, after neonatal spinal cord transection. We then showed that the prochorperazine was able to reduce spasticity after SCI *in vivo* in adult rats. This effect was blocked by pharmacological blockade of KCC2 using intrathecal injection of DIOA. Acute prochlorperazine injection *in vivo* increased the plasmalemal expression of KCC2 in the ventral horn, as revealed by means of immunohistochemistry. These data confirm that targeting KCC2 represents a valuable therapeutic strategy to reduce spasticity after SCI.

Disclosures: S. Liabeuf: None. **L. Stuhl Gourmand:** None. **P. Boulenguez:** None. **F. Gackiere:** None. **A. Viallat Lieutaud:** None. **R. Mancuso:** None. **L. Vinay:** None. **F. Brocard:** None.

Poster

627. Spinal Cord Injury: Rehabilitation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 627.06/YY6

Topic: E.09. Spinal Cord Injury and Plasticity

Support: Veterans Administration

Craig H Nielsen Foundation

Title: Forelimb rehabilitation after mild cervical injury

Authors: *E. COLLYER, K. KADOYA, P. LU, M. TUSZYNSKI;
UCSD, LA Jolla, CA

Abstract: Spinal cord injury (SCI) is a devastating condition. The specific deficits that result from injury depend on the level and severity of the injury. One major challenge for SCI repair is

to restore voluntary movement, especially hand function, after cervical injury. Rehabilitative training, alone or in combination with pro-regenerative interventions, has substantial potential to improve functional outcomes after SCI. One useful model for evaluating functional recovery in the forelimb after SCI is the C4 bilateral dorsal column lesion (DCL). This injury model removes the vast majority of corticospinal inputs onto the forelimb motor pool and creates a severe deficit in grasping behaviour, eliminating the ability of rats to retrieve food pellet rewards on a Montoya staircase task (Wang et al J Neurosci. 2011). The latter study used the Listed hooded rat, an outbred rat strain. Many studies using cell transplantation utilize inbred rat strains, such as the Fischer 344 rat. To investigate the utility of the C4 bilateral DCL model for assessing functional outcomes, we performed C4 DCL in this rat strain. Notably, the same injury model in the Fischer 344 rat resulted in only a mild deficit on pellet retrieval: from a pre-operative baseline of 17 $\pm$ 0.5 pellets, these animals exhibited a drop in the number of pellets eaten to 2 $\pm$ 0.3 when tested 7 day post-lesion. However, they spontaneously recovered to retrieval of 7 $\pm$ 0.3 pellets by 42 days post-lesion. Thus different rat strains can yield different behavioral outcomes, likely due to strain-related differences in the importance of other axonal systems in forelimb control, including the rubrospinal projection. To develop a lesion model that resulted in persistent forelimb deficits and was sensitive to rehabilitation effects, we performed bilateral C4 DCL (to remove the CST) plus right dorsal quadrant lesions (to remove the rubrospinal projection) in Fischer 344 rats. This lesion resulted in persistent deficits in right forelimb food pellet retrieval (0 pellets at 7 days to 2$\pm$0.3 pellets at 70 days). Notably, rehabilitation significantly improved the mean number of retrieved pellets on week 9 post-lesion from 1.6 $\pm$ 0.7 pellets/session in lesioned, non-rehabilitated rats to 4.6 $\pm$ 0.3 pellets/session in injured, rehabilitated rats ($P<0.05$, RM ANOVA). On anatomical analysis, lesions were consistent in size and extent. This cervical lesion model therefore offers the benefit of exhibiting a benefit from rehabilitation on the Fischer 344 rat strain. We will now test whether the combination of neural stem cell grafts and rehabilitation provide synergistic benefits in functional outcomes.

Disclosures: **E. Collyer:** None. **K. Kadoya:** None. **P. Lu:** None. **M. Tuszynski:** None.

Poster

627. Spinal Cord Injury: Rehabilitation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 627.07/YY7

Topic: E.09. Spinal Cord Injury and Plasticity

Support: British Columbia Medical Services Foundation

Canadian Institute of Health Research

Title: Changes in inter- and intra-limb coordination following locomotor training in people with spinal cord injury

Authors: *R. N. MALIK, K. PAUHL, T. LAM;
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Abstract: Background: In individuals with motor-incomplete spinal cord injury (m-iSCI), the ability to functionally ambulate is reduced, partly due to the skilled walking requirements in our everyday lives. The ability to modify gait patterns for skilled walking requires appropriate intra- and inter-limb coordination. Following SCI, coordination within a limb (intra-limb coordination) and the coordination between limbs (inter-limb coordination) can be impaired, which may contribute to the reduced ambulatory capacity among individuals with SCI. The aim of this project was to determine whether intra- and inter-limb coordination can be improved following body weight supported treadmill training with a velocity-dependent resistance applied to the lower limbs and whether improvements in coordination are related to improvements in functional walking capacity.
Methods: Individuals with chronic m-iSCI were randomly assigned to body weight support treadmill training (BWSTT) with Lokomat-applied resistance (Loko-R) or to conventional Lokomat assisted BWSTT (Control). Training sessions lasted 45 minutes, 3 times a week for 3 months. Before (baseline) and after (post-training) training, lower limb joint kinematics were recorded while participants walked on a treadmill with body weight support at their comfortable and fastest speed. Motion capture markers were used to determine, hip, knee and ankle angles during walking. Joint angles were used to determine overall range of motion and intra- and inter-limb coordination. Over-ground skilled waking capacity, measured by the Spinal Cord Injury Functional Ambulation Profile (SCI-FAP), walking speed (10-meter walk test), and endurance (6-minute walk test) were also measured at baseline and post-training.
Results: Following training individuals in the Loko-R group tended to have greater joint range of motion during gait compared to the Control group, and also tended to show improvements in lower limb coordination. The Loko-R group also showed greater improvements in over-ground skilled walking capacity compared to the control group.
Conclusion: Lower limb coordination can be improved by BWSTT with Lokomat-applied resistance. This data suggests that skilled walking capacity, range of motion and lower limb coordination could potentially be used to show improvements in motor control following SCI.

Disclosures: **R.N. Malik:** None. **K. Pauhl:** None. **T. Lam:** None.

Poster

627. Spinal Cord Injury: Rehabilitation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 627.08/YY8

Topic: E.09. Spinal Cord Injury and Plasticity

Support: FINEP 01.12.0514.00

AASDAP

AACD

Itau Bank

Title: Partial sensorimotor recovery in chronic complete spinal cord injury patients following a 24 month neuro-rehabilitation training with brain-machine interface controlled virtual and robotic gait devices

Authors: *S. SHOKUR[1], A. C. DONATI[1,2], D. CAMPOS[1], D. FISCHER[1,2], P. AUGUSTO[1], C. GITTI[1,2], G. BAO[1], E. MORYA[3,4], M. NICOLELIS[3,5,6];
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Abstract: A complete spinal cord Injury interrupts bidirectional communication between the subject's brain and body. The lesion level, severity and time since the spinal cord damage determine both the degree of sensorimotor impairment and the recovery prognosis. In the case of chronic paraplegic patients, diagnosed clinically with a complete lesion, or ASIA A (no sensory nor motor functions below the level of the injury), chances of spontaneous or induced clinical recovery are considered negligible. Here, we describe clinical results of a 24 month training protocol, based on a non-invasive closed loop brain machine interfaces, integrated with physical rehabilitation. A total of 7 SCI patients (6 ASIA A, 1 ASIA B) were followed. Patients were trained to brain control both virtual avatars in a simulated world and robotic gait trainers, using both a body-weight support system on a treadmill (Lokomat) and a custom-built exoskeleton. In addition, patients received continuous visual and tactile feedback. The latter was delivered, via a haptic display, applied to forearm skin. Tactile feedback was given in synchrony with the steps of the virtual or robotic actuators. Overall, we observed sensory improvements in all 7 of our patients, considering the perception of fine touch, pain and proprioception. Moreover, significant motor improvement, involving multiple myotomes below the level of lesion, was documented by

clinical evaluation (ASIA measurement) and validated via surface EMGs. Overall, 6 out of 7 patient had their ASIA rank updated (1 subject moved from ASIA B to ASIA C, 4 subjects moved from A to C and 1 subject moved from A to B). We propose that a long term training with closed-loop brain machine interfaces, combined with routine aided walking, may become a new rehabilitative therapy for chronic SCI patients.

Disclosures: **S. Shokur:** None. **A. C. Donati:** None. **D. Campos:** None. **D. Fischer:** None. **P. Augusto:** None. **C. Gitti:** None. **G. Bao:** None. **E. Morya:** None. **M. Nicolelis:** None.

Poster

627. Spinal Cord Injury: Rehabilitation

Location: Halls B-H

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Program#/Poster#: 627.09/YY9

Topic: E.09. Spinal Cord Injury and Plasticity

Support: Wings for Life-US-026/14

Title: Repetitive exposure to intermittent hypoxia enhances overground walking in persons with chronic incomplete spinal cord injury

Authors: ***D. M. PETERS**, H. B. HAYES, M. C. OAKLEY, R. T. HAVRANEK, R. D. TRUMBOWER;
Emory Univ., Atlanta, GA

Abstract: Improving overground walking is a highly valued goal for persons with incomplete spinal cord injury (iSCI). Repetitive exposure to short bouts of low oxygen (acute intermittent hypoxia, AIH) is a non-invasive therapy that induces locomotor recovery in rodent models and humans with iSCI. Recently, we showed that 5 consecutive days of AIH alone or with walking practice increases walking ability in persons with chronic iSCI. Despite these exciting findings, little is known about the potential long-term beneficial effects or the impact of more frequent exposures of AIH on walking recovery in persons with chronic (>1 year) iSCI. The purpose of this study was to investigate the effects of prolonged exposures to AIH on overground walking speed and endurance in persons with chronic iSCI. We hypothesize that repetitive exposures (5 times/week for 2 weeks) to modest bouts of low oxygen will enhance and prolong walking recovery in persons with chronic iSCI.
Utilizing a double-blind, counterbalanced, crossover study, four persons with iSCI (AIS D) received AIH (FIO_2 = 0.09) or normoxia (FIO_2 = 0.21) consisting of 15 episodes of 1.5 min AIH/normoxia alternated with 1 min normoxia, followed by 30 min overground walking practice. We assessed walking speed and endurance using the 10-meter and 6-minute walk tests

at baseline, treatment days 5 and 10, and at four follow-ups (out to 3.5 weeks post-treatment). Measurements of spasticity, incidences of autonomic dysreflexia and systemic hypertension were also assessed to confirm that repeat exposures to mild bouts of AIH elicits benefits without meaningful maladaptive plasticity in persons with iSCI.
AIH + walking increased overground walking speed (0.18 ± 0.02 m/s) and distance (78.1 ± 30.1 m) more than normoxia + walking (speed: -0.01 ± 0.03 m/s, endurance: -16.9 ± 27.4 m). After AIH + walking, 4 of 4 subjects exceeded the minimal clinically important difference (MCID) for walking speed (≥0.13 m/s) and 3 of 4 subjects exceeded the MCID for walking endurance (≥50 m) relative to baseline, which persisted up to 3.5 weeks in 3 subjects. AIH was well-tolerated with no increase in spasticity, no episodes of autonomic dysreflexia, and low systemic hypertension incidence rate.
Consistent with our hypotheses, AIH appears to be a safe and effective treatment strategy to enhance walking recovery in persons with chronic iSCI. When coupled with walking practice, AIH elicits prolonged improvements in overground walking speed and endurance, making AIH an attractive combinatorial adjuvant to iSCI rehabilitation. Harnessing AIH-induced plasticity to promote functional recovery may lead to safer and more efficient community walking in persons with iSCI.

Disclosures: D.M. Peters: None. **H.B. Hayes:** None. **M.C. Oakley:** None. **R.T. Havranek:** None. **R.D. Trumbower:** None.

Poster

627. Spinal Cord Injury: Rehabilitation

Location: Halls B-H

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Program#/Poster#: 627.10/YY10

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH NINDS NS054894

NIH NINDS NS072651

Title: Robot assisted rehabilitation paired with subthreshold optogenetic stimulation of motor cortex below bregma enriches cortical representation of trunk muscles in complete spinal transected adult rats

Authors: *K. A. SCHMIDT, S. F. GISZTER;
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Abstract: Due to altered sensory afferents and motor efferents, a complete T9/T10 spinal cord injury (SCI) in rats leads to reorganization of sensori-motor cortex. Neonatally transected (NTX) rats can be trained to take weight supported steps on a treadmill with the assistance of a body weight supporting robot. Robot/treadmill trained NTX rats undergo an additional cortical reorganization compared to untrained NTX rats such that the center of gravity (COG) of trunk motor areas shifts caudally, and a greater representation of trunk muscle segments below the sight of injury appear. Because this cortical reorganization is coupled with an improvement in locomotor activity in NTX rats, we asked if promoting plasticity in the trunk motor cortex below bregma using subthreshold optogenetic stimulation would affect cortical reorganization and functional changes in adult transected (ATX) rats.
Using viral delivery, we introduced Channelrhodopsin (ChR2) or a control fluorophore (EYFP) targeted to pyramidal cells of the sensorimotor cortex below bregma. With robotic assistance, some ATX rats were trained to walk on a treadmill as described above while receiving continuous 470nm light stimulation. Three groups of robot trained rats were prepared: ATX with ChR2 (ATX-ChR2, current N=6), ATX-ChR2 rats also with AAV5-BDNF injected into the lumbar spinal cord to induce hindlimb alternation (ATX-ChR2/BDNF, current N=4), and a control group (ATX-EYFP, current N=6). A cage rest group (ATX-ChR2-CR, current N=5) received constant light stimulation 20mins/day for five weeks. After five weeks, rats underwent a terminal intracortical microstimulation (ICMS) map.
A significant caudal shift in trunk representation COG was observed in robot trained ChR2 rats but not in CR rats ($p<0.05$ Wilcoxon rank-sum test). Both ChR2 robot trained groups showed significantly more information by entropy calculations in regions of cortex below bregma compared to controls ($p<0.05$ ANOVA with LSD post hoc comparison). A significant increase in lumbar trunk representation was observed in ChR2-BDNF treated rats compared to EYFP rats ($p<0.05$ Wilcoxon rank-sum test). The ChR2-BDNF group also showed more information by entropy calculations from muscles located below the site of injury, and had higher cumulative pairwise mutual information than other groups. These results show that, when paired with robot assisted rehabilitation training, optogenetic subthreshold excitation can enrich cortical trunk representation below bregma and may lead to improved functional outcome in ATX rats.
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Disclosures: K.A. Schmidt: None. **S.F. Giszter:** None.

Poster

627. Spinal Cord Injury: Rehabilitation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 627.11/YY11

Topic: E.09. Spinal Cord Injury and Plasticity

Support: Shepherd Foundation Hulse Research Fund

Title: Safety and feasibility of a pragmatic approach for augmenting fine motor training with transcranial direct current stimulation in persons with tetraplegia

Authors: *J. A. IDDINGS, S. M. CALLAHAN, E. C. FIELD-FOTE;
Crawford Res. Inst., Shepherd Ctr., Atlanta, GA

Abstract: Objectives: Restoration of upper extremity (UE) motor function is often cited as the primary rehabilitation priority among individuals with tetraplegia. Direct modulation of cortical excitability with a single session of transcranial direct current stimulation (tDCS) has been shown to improve UE function in persons with tetraplegia. However, the functional effects of a multi-session course of tDCS have not been evaluated in persons with tetraplegia using a pragmatic approach designed to closely mirror traditional UE rehabilitation. The goal of this pilot study was to determine whether tDCS was a safe and feasible adjunct to standard fine motor training (FMT) sessions in the clinical setting.
Methods: Two participants with subacute (≤ 3 months post-injury) and two participants with chronic (≥ 6 months post-injury) cervical spinal cord injury were recruited for this pilot study. Following a one week control wash-in consisting of two 1 hour sessions of FMT alone, tDCS was applied as an adjunct to inpatient/outpatient FMT for three weeks. FMT was specifically tailored to reflect the individual recovery goals of each participant. UE strength (manual muscle testing, dynamometry), sensation (Semmes-Weinstein Monofilament Test) and function (Graded Redefined Assessment of Strength, Sensibility and Prehension - GRASSP) were measured prior to the intervention (baseline), following the control wash-in (FMT) and after the three weeks of combined cortical stimulation and training (tDCS + FMT). One subacute participant also participated in a follow-up evaluation three weeks after the conclusion of tDCS + FMT.
Results: Cortical stimulation was well-tolerated by all participants with only one subject reporting a mild headache the evening after the first tDCS session. Function of the UE stimulated by tDCS improved during the study for all participants (1-4 point improvement in GRASSP quantitative total score). However, based on this small sample of subjects it is not possible to determine whether these improvements were related to cortical stimulation, as similar rates of functional and sensory gains were observed during both the control wash-in and combined cortical stimulation and training. Interestingly, the individual who participated in follow-up displayed greater functional improvements in the stimulated UE than the unstimulated UE at final evaluation.
Conclusions: This pilot study demonstrates that tDCS is a safe adjunct to FMT that is feasible for application in the clinical setting. Future studies should directly compare the multi-session effects of FMT alone vs. tDCS + FMT to determine the effectiveness of augmenting FMT with tDCS in the clinical setting.

Disclosures: J.A. Iddings: None. **S.M. Callahan:** None. **E.C. Field-Fote:** None.

Poster

627. Spinal Cord Injury: Rehabilitation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 627.12/YY12

Topic: E.09. Spinal Cord Injury and Plasticity

Support: Shepherd Foundation Hulse Research Fund

Title: Comparative effects of non-pharmacological interventions for lower extremity spasticity in persons with spinal cord injury - a pilot study

Authors: *S. ESTES, J. IDDINGS, A. HOLZWARTH, E. SANDLER, E. FIELD-FOTE; Spinal Cord Injury, Shepherd Center/Crawford Res. Inst., Atlanta, GA

Abstract: Purpose: Individuals with a spinal cord injury (SCI) often experience involuntary, reflex evoked muscle activity and stiffness associated with spasticity. While pharmacological treatments are the most often used approach to alleviate this spasticity, these treatments often have deleterious side effects. Physical therapeutic interventions offer an alternative treatment for the reduction of spasticity. However, while many different interventions have been studied, a systematic comparison of these approaches has not previously been completed. The purpose of this study was to compare four non-pharmacological interventions to assess their efficacy for spasticity reduction. Methods: Participants were individuals (n=10) with chronic SCI (≥ 1 year) who exhibited hyperreflexic responses to stretch of the quadriceps. The degree of quadriceps spasticity was objectively quantified using the pendulum test before and at two time points after (immediate and delayed: 45 min) each of 4 different physical therapeutic interventions: stretching, cyclic passive movement (treadmill-based robotic gait orthosis), transcutaneous spinal cord stimulation (tcSCS), and transcranial direct current stimulation (tDCS). A fifth intervention (peripheral stimulation, PS), consisting of a brief ramp up and down of stimulation at the knee and ankle, served as the sham control condition. A minimum 48 hour washout period was utilized between interventions. In addition to the pendulum test, electromyographic (EMG) recordings of the quadriceps, hamstrings, tibialis anterior, and soleus muscle activity were used to verify the presence of the stretch response and identify the onset of stretch-evoked muscle activation. Results: There was a significant reduction in quadriceps spasticity, as indicated by an increase in pendulum angle, for all interventions when compared to the sham control. Moreover, the decrease in spasticity from each intervention persisted for at least 45 minutes. While there was no single intervention that was significantly more effective at reducing spasticity, tcSCS showed a trend towards having a greater persistence in the reduction of spasticity than other interventions. Discussion and Conclusions: These preliminary findings indicate that single sessions of each physical therapeutic intervention can function as feasible short-term treatments for spasticity. While these findings suggest that physical therapeutic interventions may

supplement or replace standard antispastic medications, future studies should directly compare the antispastic effects of these physical therapeutic interventions to those of the antispastic medications.

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Poster

627. Spinal Cord Injury: Rehabilitation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 627.13/YY13

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH R01NS079569

Title: Leveraging activity and guidance to promote sprouting of direct connections between corticospinal tract axons and spinal motoneurons in PlexinA1 knockout mice.

Authors: *J. KALAMBOGIAS[1,2], Z. GU[3], Y. YOSHIDA[3], J. MARTIN[1,2];
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Abstract: The corticospinal tract (CST) is critical for skilled and voluntary movements. Damage to the CST after stroke or SCI results in motor impairments. Most injuries are incomplete and promoting spared CST connections after injury is an important strategy for CST repair. In rodents, CST connections are made primarily with contralateral spinal interneurons. In many monkey species and humans, the CST makes mostly contralateral and some ipsilateral projections to spinal cord interneurons and, importantly, motoneurons. Thus rodent models fail to capture some of the key features of the CST in humans. Mice with conditional forebrain deletion of the gene for Plexin A1 (PlexA1KO), have an abundance of direct connections between the CST and motoneurons, as well as ipsilateral descending projections in white matter territories similar to primates and humans (Gu et al, submitted; Kalambogias et al; SFN abstracts 2015), making this mouse potentially an important model to study CST repair. The effect of gene deletion on CST connections is attributable to eliminating axon repulsion and maintenance of exuberant projections during development due to loss of CST PlexinA1-Semaphorin6D signaling. Here we used this novel mouse model with a CST that shares key features of the human CST to determine if M1 electrical stimulation, which we have shown in rats promotes CST sprouting, can be used to establish more corticomotoneuronal (CM) connections and

stronger M1 to muscle throughput. We assayed for CM connections using M1 stimulus-triggered averaging and confocal microscopy. To promote activity-dependent sprouting, we chronically stimulated M1 unilaterally for 10 days (6 hours/day; Brus-Ramer et al 2007). In adult uninjured PlexA1 KO mice, anterograde tracing revealed increased CST axon length and varicosities within cervical motor pools in stimulated compared to non-stimulated mice. Stimulation also increased projections to the intermediate zone and, generally, to the ipsilateral side. Morphological analyses are in progress to determine if there were similar changes in putative CST synapses on motoneurons and interneurons. The anatomical changes were paralleled by a substantially increased capacity for M1 to activate forelimb muscles. Our findings show that activity-dependent sprouting takes advantage of the reduced repulsive effects of PlexinA1-Sema6D signaling to increase CM connections, as well as interneuron connections and ipsilateral CST projections. That CST neuron activation can lead to the formation of new CM connections in this model further suggests that PlexinA1-Sema6D signaling is important in the maturity in specifying CST connections.

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Poster

627. Spinal Cord Injury: Rehabilitation

Location: Halls B-H

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Program#/Poster#: 627.14/YY14

Topic: C.09. Brain Injury and Trauma

Support: ANR agence Nationale de la Recherche

Title: Effects of different rehabilitative locomotor trainings on the innervation of lumbar motoneurons in adult rats with complete spinal cord transection.

Authors: *H. BRAS[1], M.-C. MORIN, Jr[2], O. ALLUIN[4], M.-P. COTÉ[5], S. ROSSIGNOL[6], F. BROCARD[3];
[1]CNRS, Marseille Cedex 05, France; [2]INT, [3]CNRS, AMU, Marseille, France; [4]CNRS UMR 7287, AMU, Marseille, France; [5]Drexel university, Philadelphia, PA; [6]Faculté de medecine, Montreal, QC, Canada

Abstract: More than 250,000 traumatic spinal cord injuries are reported every year worldwide, causing paralysis of the lower limbs in 53 % of the cases. Locomotor training has been shown to be efficacious in the progression of neurologic rehabilitation. It is founded on the principle of activity dependent plasticity but the understanding of its mechanisms remains poorly investigated. In Wistar female adult rats with complete spinal cord transection (SCT), we

compared the effect of 3 different types of locomotor training on the innervation of lumbar motoneurons (MNs). The training procedures, which started one week after SCT and lasted 10 weeks, were as follows: 1) Passive training of bicycling exercise (BE rats, Coté et al., 2014); 2) Imposed treadmill training with perineal stimulation (PS rats, Alluin et al. 2015); 3) Combination of spontaneous moving in enriched environment, treadmill training and physiotherapy, completed after 8 weeks by 5-HT_2 agonists treatment (UOS rats, Bras et al., 2013). By means of immunocytochemistry we analyzed: 1) Occurence of cholinergic (VACHT) and glutamatergic (VGLUT1) excitatory inputs. 2) Occurrence of inhibitory GABA and Glycine inputs (VGAT). 3) Density of P-boutons (GAD65, pre-synaptic inhibition) on primary sensory afferents (VGLUT1). More than 450 MNs with similar diameters were selected in L3-L4 lumbar segments. In intact rats, VGLUT1 and VACHT axon terminals were large boutons (5 μm2), sparsely distributed on the MN membrane (3 and 5 per 100 μm). VGLUT1 received a mean number of 1.6 p-boutons. VGAT terminals were smaller boutons (2.4 μm2), 7 times more numerous than VGLUT1. In BE rats, a significantly increase of the size of VGLUT1 terminals suggested an increased excitability of MNs in absence of changes in the density and size of VACHT and VGAT terminals as well as p-boutons. In PS rats the density of VGLUT1 and the size of VACHT boutons significantly decreased whereas the size of VGAT terminals and p-boutons were significantly reduced, suggesting a decrease of both excitatory and inhibitory innervations. In UOS rats, a significant increase of the size of cholinergic excitatory inputs as well as VGAT inhibitory inputs, together with an increased density of the p-boutons, suggested a reinforcement of both excitation and inhibition. In sum, different training procedures of SCT rats result in specific changes of the innervation of lumbar MNs. This results show that sub-lesional lumbar premotor interneurons are able of adaptive plastic changes of their innervation to adjust the balance between excitation and inhibition and adapt to alterations of the integrative properties of MNs occurring after SCI.

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Poster

627. Spinal Cord Injury: Rehabilitation

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 627.15/ZZ1

Topic: E.09. Spinal Cord Injury and Plasticity

Support: AIHS

CIHR

Title: Gait kinematic analysis in people with incomplete spinal cord injury after arm and leg cycling training

Authors: *R. ZHOU[1], L. ALVARADO[1], O. SHAW[3], R. OGILVIE[1], S. CHONG[1], V. MUSHAHWAR[1,2];
[1]Ctr. for Neurosci., [2]Div. of Physical Med. & Rehabil., Univ. of Alberta, Edmonton, AB, Canada; [3]Univ. of Toronto, Toronto, ON, Canada

Abstract: The goal of this project is to develop an intervention for improving walking after incomplete spinal cord injury (iSCI). Arm activity can significantly modulate the performance of the legs in various types of rhythmic locomotor activities including walking and cycling, which are generated by a common spinal network. Despite this knowledge, current rehabilitation interventions for improving walking do not actively involve the arms.
Therefore, we proposed the combined use of arm and leg cycling in a 12-week training paradigm for people with iSCI to improve ambulation. Thirteen volunteers with chronic iSCI were divided in 2 groups: arm & leg cycling (A&L) group and leg only cycling (Leg) group as control. We investigated: 1) the role of arms in the improvement of walking; and 2) the transfer of the effects of recumbent cycling to upright walking.
There was no significant difference between the two groups in walking speed or walking distance at the pre-training stage. Compared to pre-training, the 10-meter walking speed test showed significant improvements of 0.10 to 0.62 m/s in the A&L group, and 0.02 to 0.21 m/s in the Leg group. The A&L group improved significantly more in normalized speed change than the Leg group ($p=0.03$). Similarly, the 6-minute walking distance test showed post-training improvements of 29.83 to 292.84 m in the A&L group, and -7.04 to 61.09 m in the Leg group, although the difference in improvements between the two groups was not significant ($p=0.38$). Spatiotemporal kinematic parameters were measured (i.e. double support, step length, swing time and stance time) to evaluate the quality of over-ground walking. Participants were instructed to walk on a 6-m long track at self-selected speeds before and after training. After training, the A&L group showed significant improvements in most of the measures, whereas the Leg group only had significantly faster self-selected walking speed after training. Hip-knee cyclograms illustrated the angular excursions of the two joints within each gait cycle. Significantly higher cycle consistency of the hip-knee joint movement ($p=0.016$) and larger area within the cyclogram ($p=0.042$) were found. Furthermore, the stronger side in the A&L group had a significant increase in the consistency coefficient of 0.06 ± 0.02, but no significance in consistency was found in the Leg group.
This study suggests that non-gait-specific neurorehabilitation techniques could be translated into improved ambulation. More importantly, the findings provide strong support for active engagement of the arms in lower limb rehabilitation for more effective restoration of leg function.

Disclosures: R. Zhou: None. **L. Alvarado:** None. **O. Shaw:** None. **R. Ogilvie:** None. **S. Chong:** None. **V. Mushahwar:** None.

Poster

627. Spinal Cord Injury: Rehabilitation

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: Grant-in-Aid for JSPS Fellow

Magnetic Health Science Foundation

Title: The effect of trans-spinal static magnetic field stimulation on the corticospinal excitability of upper limb muscles

Authors: *K. NAKAGAWA[1,2], K. NAKAZAWA[1];
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Abstract: Neuromodulation techniques to spinal neural circuits can be applied to enhance rehabilitation for individuals with spinal cord injury patients (Herman et al. 2002; Harkeme et al. 2011). To date, some techniques such as transcutaneous spinal direct current stimulation (Lamy et al. 2012) or repetitive trans-spinal magnetic stimulation (Gerasimenko et al. 2010) have been utilized to non-invasively modulate spinal neural activity. Recently, transcranial static magnetic field stimulation (tSMS) has been demonstrated to modulate cortical function as one of the most non-invasive and painless neuromodulation tools (Oliviero et al. 2011; 2015). In the present study, we investigated the effect of tSMS applied over the spinal cord on the corticospinal excitability for testing a potential new technique to modulate spinal neural activity. To obtain static magnetic field, we utilized a cylindrical neodymium magnet (50 mm × 30 mm) with a magnetic intensity at the surface of the magnet of 0.46 T. We applied tSMS and sham stimulation with putting the magnet or non-magnetic steel cylinder over the cutaneous surface of the neck (C8 level) for 15 minutes to healthy subjects. Motor evoked potentials (MEPs) were recorded from muscles (target: first digital interosseous (FDI)) in right upper limb by single transcranial magnetic stimulation (TMS) of the left motor cortex. MEPs were recorded before, during and after tSMS and sham stimulation. All subjects participated in two experimental conditions (tSMS and sham stimulation) on separate days. The experiments were performed in a double-blinded design. Results showed 20~30% reduction of the MEPs on average during and after tSMS. Two-way repeated ANOVA showed a significant main effect of condition (tSMS and sham). It indicated that the corticospinal excitability in upper limb muscles was decreased by the tSMS compared to sham stimulation. This result suggests that tSMS is able to be a future neuromodulation tool for individuals with spinal cord injury.

Disclosures: K. Nakagawa: None. **K. Nakazawa:** None.

Poster

627. Spinal Cord Injury: Rehabilitation

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Topic: E.09. Spinal Cord Injury and Plasticity

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The Grainger Foundation

Title: Characterization of stimulation-evoked muscle activity via intrapsinal microstimulation in a swine model

Authors: *J. S. CALVERT, P. J. GRAHN, A. A. MENDEZ, K. E. BENNET, I. A. LAVROV, K. H. LEE, J. L. LUJAN;
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Abstract: Introduction: Chronically paralyzed spinal cord injured (SCI) patients have shown the ability to generate voluntary step-like movements via electrical spinal stimulation. Intraspinal microstimulation (ISMS) is a technique which has shown promise in restoring motor function by activating specific muscle groups while minimizing fatigue. We have developed a large animal swine model of spinal stimulation to take advantage of the anatomical similarities between human and swine spinal cords and to facilitate the development of spinal cord stimulation technologies. However, the intervertebral and intraspinal locations of motor pools that drive muscle activity responsible for limb function need to be elucidated before this model can be used to develop technologies for functional restoration. Therefore, we set out to characterize these motor-evoked responses using ISMS to create a functional atlas of spinal-evoked responses by recording muscle activity in the hind limbs of swine.
Methods: Swine (n=6 female) hind limb flexion, extension, abduction, and adduction movements were evoked using intraspinal stimulation (50 Hz, 2 s, 200 μA) at different depths, separated by 250 microns, and following a dorsoventral implantation trajectory. During stimulation, electrical activity from the biceps femoris, gastrocnemius, gluteus medius, gracilis, tibialis anterior, and soleus muscles was recorded using intramuscular electromyography (EMG). Spatial mapping of muscle activation was achieved by co-registering subject-specific post-mortem magnetic resonance imaging (MRI), computed tomography (CT) scans, and stereotactic electrode coordinates in a single coordinate system.
Results: As the electrode was advanced into ventrally-located motor regions of the spinal cord, the motor-evoked muscle responses varied, typically displaying an increasing response as the electrode approached the ventral limit of the trajectory. A functional atlas of the swine spinal cord was established via co-registration of the CT and MRI scans across stimulation parameters and across animals.

Conclusions: This functional atlas established will permit targeted stimulation of specific hind limb muscles via stereotactic delivery of intraspinal electrodes for targeted activation of hind limb muscles in chronic SCI models. This work will be used to evaluate responses in a chronic model of SCI, which can be used to optimize clinical stimulation techniques.

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Poster

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NYS DOH C30860GG

Craig Neilsen Foundation 261214

Title: Chronic theta burst electrical stimulation of rat motor cortex promotes CST outgrowth and M1-to-muscle connection strength

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Abstract: SCI interrupts connections between the brain and spinal cord. An important strategy for promoting motor function after SCI is to promote sprouting of the spared axons of descending motor pathways. We focused on the corticospinal tract (CST), which is essential for skilled voluntary movements. We showed that electrical stimulation of the motor cortex (M1) to activate CST neurons enhances CST outgrowth and promotes motor recovery. We recently showed that combined chronic M1 and trans-spinal direct current stimulation (tsDCS), 30 minutes/day for 10 days, results in robust CST outgrowth and motor recovery after pyramidal tract lesion (Song et al., 2016). In that study we used intermittent theta burst stimulation (iTBS), which has the same pulse pattern as the commonly-used magnetic neuromodulatory stimulation in humans for producing corticospinal system plasticity. Electrical iTBS in rats produced strong short-term enhancement of evoked muscle activation. Importantly, only 30 minutes of daily stimulation was effective in producing durable, functional plasticity. The goal of the present

study is to determine the differential contributions of iTBS M1 stimulation and and tsDC on CST outgrowth and M1-to-muscle connection strength in rats. Chronic stimulation, between day 10 and day 20 after unilateral pyramidal tract lesion (PTX), was delivered for 30 minutes daily. We examined 4 animal groups: injury only; injury+tsDC; injury+iTBS; injury+combined iTBS and tsDC. Cervical DC stimulation was cathodal and was applied using skin electrodes (trans-spinal). To date, we have analyzed the effects of iTBS only. The contributions of spinal DC is in progress. iTBS produced significant CST sprouting into the ipsilateral (denervated) intermediate zone and motor pool compared with the injury-only animal group. We assayed M1-to-muscle connection strength by constructing muscle response recruitment curves that show the increase in muscle evoked responses to progressively larger amplitude M1 stimulation. Compared with injury only, iTBS substantially enhanced the muscle response recruitment curve. This enhancement was paralleled with an expansion of the ipsilateral cortical motor map of the impaired/denervated wrist and elbow. Our findings show that a remarkably brief daily period of iTBS of M1 is capable of producing robust CST axonal outgrowth and stronger M1-to-muscle connections. This suggests that M1 neuromodulatory approaches commonly used in humans could produce structural plasticity in the spinal cord and further informs activity-based therapies for promoting function after injury.

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Poster

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Title: Delayed treatment of forelimb flexor muscles with an AAV encoding human Neurotrophin-3 normalizes muscle afferent connectivity, treats abnormal proprioceptive reflexes, reduces forelimb spasms, and improves walking after bilateral corticospinal tract injury in rats

Authors: *L. D. MOON[1], T. H. HUTSON[2], S. B. MCMAHON[1], C. KATHE[1];
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Abstract: CNS injury often causes spasticity and disability. We now show that bilateral transection of the corticospinal (CST) tracts in the pyramids causes flexor spasms in the forelimbs, hindlimbs and tail that are easily quantified from movies of freely moving rats in the open field. Fortnightly H-reflex testing confirmed that naïve rats show rate-dependent depression of a monosynaptic reflex of a flexor forelimb muscle whereas rats with bilateral pyramidotomy exhibit less rate-dependent depression (i.e., hyper-reflexia). Polysynaptic reflexes between antagonist muscles were increased after CST injury, which may cause co-contraction. Corticospinal tract injury slightly reduced grip strength and caused mild forepaw hypersensitivity (Von Frey). Walking was impaired on a horizontal ladder with irregularly spaced rungs.
Injection of AAV1 encoding human prepro neurotrophin-3 (NT3) unilaterally into forelimb flexor muscles reduced all these signs of spasticity (relative to AAV1-GFP). Intramuscular NT3 progressively reduced forelimb flexor spasms in the open field and normalized proprioceptive monosynaptic H-reflexes of a flexor forelimb muscle. This is consistent with expression of TrkC receptors in proprioceptive muscle afferents. NT3 also normalized polysynaptic reflexes to muscles supplied by the ulnar nerve but only those involving afferents from injected muscles (e.g., synergist flexor muscles and not antagonist extensor muscles). NT3 normalised the pattern of excitatory synapse-like boutons from primary afferents upon motor neurons. NT3 also normalized the pattern of vGAT+ boutons on vGluT1+ afferents, indicating that pre-synaptic inhibition may have been restored. NT3 also normalized the level of the KCC2 ion transporter in motor neuron membranes, which may indicate more-normal neuromuscular excitability. Finally, NT3 was transported in afferents from injected muscles to the DRG. RNAseq of cervical DRG identified mRNAs and miRNAs whose levels were dysregulated by CST injury but were normalized by NT3 treatment. Finally, NT3 treatment did not cause cutaneous hypersensitivity (pain). Thus, whereas existing therapies for spasticity are symptomatic, NT3 treats many underlying causes of spasticity as well as its symptoms.
These findings are exciting because (1) we administered NT3 in a clinically relevant time frame and by a straightforward route. (2) Recombinant NT3 is safe and well-tolerated in five Phase I and II clinical trials (unlike NGF). (3) The world's first gene therapy (Glybera) involves i.m. injection of an AAV1 encoding a different transgene, which paves the way for NT3 as a therapy for CNS injury.

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Poster

627. Spinal Cord Injury: Rehabilitation

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Topic: E.09. Spinal Cord Injury and Plasticity

Title: The effects of robotic gait training on seated balance and postural control for individuals with a complete spinal cord injury

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Abstract: Background: Postural control is the ability to maintain or restore a state of balance during any posture or activity. It involves the coordination of the trunk, lower and upper limbs, and head along with inputs from multiple sensory systems. Many people with a spinal cord injury (SCI) at or above the level of T6 experience impaired postural control while seated due to a loss of innervation of the trunk muscles. Improving seated postural control is particularly important in SCI wheelchair users because it increases independence with activities of daily living, facilitates functional mobility, and enhances quality of life. Robotic exoskeletons facilitate gait training for those with neurological impairments, but it remains unknown how they engage postural muscles to control balance during walking. If training with robotics challenges dynamic postural control, secondary benefits of training may include the transfer of improved postural control to seated balance activities. The purpose of this study is to examine changes in seated balance control in people with motor-complete SCI after gait training with robotic exoskeletons.
Methods: Individuals with motor-complete SCI at or above the level of T6 (n= 4) were randomly assigned to complete 3 training phases with robotic exoskeleton gait devices, either Ekso-Lokomat-Ekso or Lokomat-Ekso-Lokomat. Each phase involves 10 sessions completed over 3 weeks with up to 45 minutes of walking per session. During these sessions, we focus on increasing comfortable walking speed. We evaluate seated balance control before and after each training phase. The center of pressure (COP) variability during eyes open (EO) and eyes closed (EC) conditions, and total distance during a limit of stability (LOS) test is calculated from a forceplate.
Results: Subject 1 reduced COP sway after phase 1 Ekso-training (EO= 3.17 to 1.66mm, EC= 4.23 to 3.48 mm). COP sway increased to 2.48mm during EO and remained at 3.47mm for EC after phase 2 Lokomat-training. LOS total distance was 323 mm at baseline, 385mm after phase 1, and 319mm after phase 2. Subject 2 is currently in phase 2. After Ekso-training in phase 1, Subject 2 reduced COP sway (EO= 4.36 to 3.09mm, EC= 6.96 to 4.23mm) and improved LOS

total distance from 194.6mm at baseline to 217.6mm after phase 1. Subject 3 and 4 are currently in phase 1.
Conclusions: Our preliminary findings show that gait training with robotics may improve seated balance control following SCI. This work will be important to understand how balance control mechanisms are challenged during gait training with robotics.

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Poster

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH K12 HD055929

Brooks-PHHP Research Collaboration

Craig H. Neilsen Foundation

Title: The effects of acute intermittent hypoxia on diaphragm activation and respiratory function in an individual with spinal cord injury

Authors: *E. J. FOX[1,3], A. D'ALESSANDRO[3], T. SUTOR[1], A. KERWIN[4], H. WEISS[3], G. JACOBS[3], K. MAHER[3], M. HOEFNAGEL[3], G. S. MITCHELL[2], D. D. FULLER[2];
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Abstract: Background: Individuals with cervical spinal cord injuries (SCIs) often have severe respiratory impairments and require ventilatory support. Rhythmic diaphragm activation via intramuscularly placed wires (i.e. “pacing”) is a relatively new approach for sustaining ventilation after acute SCI, and the intramuscular wires also enable recording of diaphragm electrical activity. Acute, intermittent exposure to moderate (and safe) levels of hypoxia (IH) is a therapeutic approach that can trigger spinal neuroplasticity and increases in respiratory and somatic motor output in both animals and humans with SCI. However, the direct impact of acute IH on diaphragm activation has never been examined in a person with SCI. In addition, the potential for IH to modulate respiratory function has not been explored in individuals with severe respiratory impairment after SCI. Accordingly, the purpose of this case report is to describe the effects of IH on respiratory function and diaphragm activation in an adult with SCI and dependence on diaphragm pacing. Methods: A 46 year-old male with complete C5 SCI (AIS A)

completed eight, 1-minute bouts of IH. Each bout consisted of breathing a mildly hypoxic gas mixture (10-15% oxygen) through a face mask, interspersed with 1-minute periods of room air breathing. Blood oxygen saturation was monitored. Maximal expiratory and inspiratory pressures as well as diaphragm electromyograms were recorded prior to and 45-minutes after completion of IH. Diaphragm muscle activation was quantified based on the peak amplitudes of activation recorded during a maximal inspiratory effort. Results: During the bouts of IH, blood oxygen saturation decreased from 96 to 86% for brief intervals and then rebounded. This response is consistent with prior reports of IH in adults with SCIs. Following eight bouts of IH, maximal expiratory pressure increased 45% from 11.5 to 16.7 cmH20, while maximal inspiratory pressure increased 18% from 22.3 to 26.3 cmH20. Peak amplitude of diaphragm electromyograms recorded from intramuscular wires increased 6%. Conclusions and Discussion: The outcomes demonstrate that acute IH is feasible and potentially beneficial in individuals with severe SCIs and dependence on diaphragm pacing. The increased respiratory pressures and diaphragm activation following IH are consistent with facilitation of respiratory synaptic pathways, as demonstrated in numerous animal studies. To our knowledge this is the first time IH has been applied to an individual with a diaphragm pacer and severe respiratory impairment. The outcomes suggest great therapeutic potential of IH to trigger plasticity and motor recovery in severe cases of SCI.

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Poster

627. Spinal Cord Injury: Rehabilitation

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Title: Deep brain stimulation of the mesencephalic locomotor region in incomplete spinal cord injury in rats

Authors: *A.-S. HOFER[1], M. P. SCHNEIDER[1], A. K. ENGMANN[1], A. C. MOSBERGER[1], L. C. BACHMANN[2], N. RUSSI[1], S. IMOBERSTEG[1], H. KASPER[1], M. E. SCHWAB[1]; [1]Brain Res. Inst., Univ. and ETH Zurich, Zurich, Switzerland; [2]The Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Incomplete spinal cord injury, characterized by sparing of some fibers and parts of tracts still connecting the brain with the spinal cord caudal to the lesion, affects millions of people worldwide. Loss of motor control and locomotion is common, also in patients who retain some degree of leg muscle innervation. A small group of neurons in the pedunculopontine and cuneiform nuclei of the midbrain tegmentum, the mesencephalic locomotor region (MLR), are well known to initiate and control locomotion in the spinal cord predominantly indirectly via ipsi- and contralateral reticulospinal tracts. Electrical deep brain stimulation (DBS) of the MLR using the brain´s intrinsic motor command circuits was shown to be a novel treatment strategy to induce recovery of locomotor functions in rats with subtotal spinal cord lesions. In order to investigate the long-term effects of DBS of the MLR on hindlimb motor function and cortical control, we stereotactically implanted DBS electrodes unilaterally into the MLR of rats, which afterwards received extensive thoracic spinal cord injuries. Detailed functional analyses of joint movements and limb kinematics during overground locomotion with (DBS-ON) and without stimulation (DBS-OFF) were performed. MLR-DBS acutely enables close to normal locomotion in rats with functional deficits resembling those observed in patients able to stand, but with very limited walking abilities. MLR-DBS enhances particularly speed and frequency of hindlimb movements during walking as well as under gravity-released conditions during wading and swimming. Importantly, rats avoid clashing into walls or obstacles, suggesting that cortical control is preserved at low and medium intensities during acute stimulation. The long-term functional and anatomical effects of DBS of the MLR are currently under investigation. MLR-DBS may represent a novel strategy not only to acutely awake locomotor activity, but also to train severely locomotor impaired patients to permanently reach a higher level of functionality.

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Poster

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Title: Exercise modulates sensory processing through chloride homeostasis after SCI.

Authors: *G. CARON, S. CHOYKE, B. DUFFY, J. WILSON, M.-P. COTE;
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Abstract: Spinal Cord Injury (SCI) undeniably decreases the amount of activity performed by an individual. Activity-based therapies are part of comprehensive rehabilitation strategies, but the mechanisms underlying the observed functional improvement after SCI remains poorly understood. Normal $GABA_A$ receptor function is critically dependent on chloride homeostasis, which is largely determined by the relative expression of two chloride transporters, the outward-rectifying KCC2 and the inwardly-directed NKCC1. SCI decreases KCC2/NKCC1 ratio in dorsal horn neurons and lumbar motoneurons with a consequent increase in intracellular Cl^-. This disruption in chloride homeostasis after SCI leads to neuropathic pain and hyperreflexia/spasticity. We have showed that chloride homeostasis can be restored by physical activity with consequent improvement in reflex modulation and also decrease hyperreflexia. The regulation of chloride homeostasis is not only involved in the regulation of motor output, but is also involved in sensory processing. A notable exception to chloride transporters expression takes place in primary afferents neurons. Because KCC2 is not expressed in primary afferents neurons located in DRGs, chloride levels remain high and $GABA_A$ receptors activation induce a primary afferents depolarization (PAD). Here, we explore the role of NKCC1 expression level on the level of presynaptic inhibition after SCI and the beneficial effects of physical activity on its recovery. NKCC1 expression will be quantified using PCR and Western blot analysis of L3-L5 DRGs and will be correlated to the level of presynaptic inhibition measured as dorsal root potentials in step-trained rats and untrained rats. Our results suggest that SCI decreases NKCC1 expression in DRG neurons associated with a decrease in presynaptic inhibition. Step-training returned NKCC1 levels and improved presynaptic inhibition without increasing neuropathic pain. Differential results obtained from proprioceptive and nociceptive afferents will be presented.

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Poster

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: SNF Dynamo 315230_149902

Title: Stable and biocompatible intraneural electrodes alleviate leg motor deficits after spinal cord injury

Authors: *S. M. WURTH[1], M. CAPOGROSSO[1,3], S. RASPOPOVIC[1,3], J. GANDAR[2], A. CUTRONE[3], N. KINANY[1], G. FEDERICI[1], Q. BARRAUD[2], N. PAVLOVA[2], G. COURTINE[2], S. MICERA[1,3];
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Abstract: Neural interfaces enable reading from and writing into the peripheral nervous system (PNS). Their stimulation capabilities have opened opportunities to restore lost sensation and alleviate motor deficits. However, incomplete characterisation of the long-term usability and bio-integration of intra-neural electrodes has restricted their clinical applications. Here, we conducted a longitudinal assessment of the stability, functionality, and biocompatibility of polyimide-based intra-neural electrodes that were inserted in the rat sciatic nerve of healthy or spinal cord injured rats for up to six months. Regular neurophysiological assessments demonstrated the ability to recruit extensor and flexor muscles of the ankle selectively throughout the experiments with electrical stimulation properties stabilizing three to four weeks after the implantation. The time course of these adaptations coincided with the progressive development of a fibrotic capsule around the electrodes. Despite this foreign body reaction, the selectivity remained stable over the following months. These functional properties supported the development of control algorithms that modulated the forces produced by ankle extensor and flexor muscles with high precision. We exploited this selectivity in a rat model of severe spinal cord injury to develop hybrid neuromodulation strategies combining peripheral and epidural electrical stimulations applied over lumbar segments. Continuous epidural electrical stimulation (EES) is known to enable locomotion of paralyzed rats. The additional, closed-loop control of peripheral nerve stimulation allowed the selective and graded tuning of key gait features, which enabled the rats to walk overground and climb staircase despite the interruption of brain input. This complementary EES-PNS neuromodulation therapy opens up novel opportunities to enable and restore motor control after neurological disorders.

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Poster

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Topic: E.09. Spinal Cord Injury and Plasticity

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Title: Exposure to acute intermittent hypoxia enhances upper extremity function in individuals with spinal cord injury

Authors: *M. S. SANDHU, B. AFSHARIPOUR, G. RASOOL, W. Z. RYMER;
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Abstract: Most spinal cord injuries (SCIs) are incomplete, however spontaneous plasticity, mediated via the spared spinal pathways is insufficient to restore normal function. One approach to augment plasticity in spinal networks is via intermittent brief exposure to mild hypoxia (also known as acute intermittent hypoxia or AIH). AIH induces rapid neuroplasticity, and has been shown to enhance volitional lower limb function in persons with incomplete SCI. Whether AIH induced neuroplasticity is equally prevalent in spinal motor pathways regulating upper limb musculature is not known. Accordingly, in ongoing studies we are testing the hypothesis that AIH will augment upper limb neuromotor function in humans. In two sets of experiments, we quantified the effect of a single session of AIH (15, 90-second episodes of 10% oxygen) on arm and hand function. First, we measured isometric flexion force at the elbow during maximal voluntary contraction (MVC) in three subjects (2 able-bodied, and 1 incomplete cervical SCI). Muscle activity from the biceps brachii was also recorded using a 128 channel high density EMG grid. The root mean square (RMS) value for each channel was calculated to generate muscle activity maps. MVC was done before, immediately after, and 60-minutes post-AIH. Sham normoxia trials were done one week later in the same individuals. We found that isometric MVC at the elbow increased by 45±5% in the two able bodied individuals, and by 30% in the SCI subject, at 60 minutes post-AIH. This increase in strength correlated with increased activation of biceps brachii. Average RMS value increased from 198±11uV to 350±3uV, and from 351uV to 392uV following AIH, in the healthy subjects and SCI patient, respectively. Sham AIH resulted in no change. In the second set of pilot experiments, we are quantifying the time-course of outcome improvement and decay following a single session of AIH on grip and pinch strength. A baseline assessment was made before administration of AIH, immediately after AIH, and every 30 minutes for up to 5 hours in three individuals with SCI. We found that the mean grip strength increased by 26±4% at 60 minutes post AIH, and the effect of AIH persisted for up to 3 hours. In comparison, there was a 5±3% change after normoxia in the same individuals after sham AIH.

These preliminary observations demonstrate the potential of AIH to enhance motor strength and upper limb function in persons with chronic incomplete SCI.

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Poster

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Alberta Innovates – Health Solutions sponsored Project SMART team

Title: Functional map of intraspinal microstimulation in the lumbar spinal cord of non-human primates

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Abstract: **Introduction:** The overall goal of this study is to prepare intraspinal microstimulation (ISMS) for testing in humans. ISMS is an electrical stimulation method developed in our lab for restoring standing and walking after spinal cord injury. It involves the implantation of arrays of penetrating electrodes in the lumbosacral spinal cord and the passing of minute electrical currents to activate networks of motoneuronal pools that evoke coordinated leg movements. Results from animal studies suggested that ISMS may produce better functional outcomes than clinically available interventions, and justified its translation to humans. The functional organization of the motoneuronal networks targeted by ISMS is well studied in cats; however, it is not directly known for humans. Yet, this information is necessary in order to translate ISMS from cats to humans. Therefore, as an intermediate step, this study focused on mapping the ISMS targets in non-human primates.

Methods and Results: Experiments were conducted in three rhesus monkeys under pentobarbital anesthesia. A laminectomy was performed to expose the lumbosacral enlargement, and the ventral horns were mapped by stimulation through a microelectrode. The stimulation parameters were: 50 Hz frequency, 0.5s long pulse trains, 200μs pulse width and amplitudes ranging from 10μA to 100μA. The electrode was mounted in a micromanipulator and moved in steps of 0.5mm, 0.5mm and 2mm, in the mediolateral, dorsoventral and rostrocaudal directions, respectively. The animal's leg ipsilateral to the side of spinal cord stimulation was suspended to allow visualization of the evoked movements. At each location, leg movement and stimulation threshold were documented and at selected locations, kinematics, force and EMG signals were also recorded. In three animals, a total of 117, 218 and 112 locations were stimulated in a 35.3 ± 1.2 mm long region of the lumbosacral enlargement. Of those, 43.4 ± 5.1% resulted in movement production. The majority of these responses had stimulation thresholds ≤10μA. The functional organization of the motoneuronal pools obtained in these experiments was consistent between animals and similar to that of cats.
Conclusions: The overall functional organization of the ISMS targets is similar in the lumbar spinal cords of cats and rhesus monkeys. Therefore, we anticipate that the functional map in humans will be similar to that of the monkeys and cats. These findings will guide the target locations for the first exploratory testing of ISMS in humans.
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Poster

627. Spinal Cord Injury: Rehabilitation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 627.27/ZZ13

Topic: E.09. Spinal Cord Injury and Plasticity

Support: PVA Research Foundation Grant #3068

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NIH SBIR R43EB018232

Title: Postural control in individuals with complete paralysis using non-invasive spinal cord stimulation

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Abstract: Previous findings demonstrated that epidural stimulation over the lumbosacral spinal cord can activate the spinal neural networks in individuals with severe spinal cord injury (SCI), and facilitate standing. Given that one of the results using epidural stimulation was recovery of standing, the overall objective of this study was to determine the effects of painless transcutaneous electrical spinal cord stimulation, or painless enabling motor control (pcEmc) combined with motor task-specific therapeutic activity during standing on the neurophysiological, clinical, and functional outcomes in individuals with a severe SCI. Thus far, we evaluated the acute and cumulated effects of pcEmc and standing in five participants with chronic motor and sensory complete SCI. Without spinal cord stimulation, full assistance during standing was required in all participants. In fact, they were able to maintain upright posture only because of the trainers' assistance at the knees and hips, and because of extensive bearing on their arms. The combination of stimulation features most critical for facilitating effective standing with pcEmc were: a) both distal and proximal leg muscles must be activated, with predominant activation of extensors; b) the stimulation intensity should be sufficient to evoke motor threshold responses in non-weight-bearing condition; c) frequencies between 10 to 20 Hz should be used. These features of pcEmc applied at multiple low thoracic and lumbosacral levels required the least amount of assistance to support the participants. Some periods of self-generated standing occurred within the first training session. Three participants regained the ability to stand in the presence of stimulation, without assistance applied to the knees or hips while there was only light contact with the finger tip on a handlebar. In summary, our results demonstrate the feasibility and effectiveness of transcutaneous electrical spinal cord stimulation as a means to regain dynamic standing ability even after complete paralysis. As with the invasive epidural stimulation approach, there can be an effective reawakening of relatively dormant spinal networks that can relearn how to stand. Such non-invasive intervention could be a viable clinical approach for functional recovery after paralysis of different severity among a broad SCI population.

Disclosures: D. Sayenko: None. **R. Edgerton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroRecovery Technologies. **Y. Gerasimenko:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroRecovery Technologies.

Poster

627. Spinal Cord Injury: Rehabilitation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 627.28/ZZ14

Topic: E.09. Spinal Cord Injury and Plasticity

Support: Wings for Life

NIH RO1 NS095366

Title: Plasticity of spinal rhythm generating interneurons after spinal cord injury

Authors: S. BIBU, N. HA, L. YAO, *K. J. DOUGHERTY;
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Abstract: Neural circuitry generating rhythm and pattern components of locomotion is located in the thoracolumbar spinal cord. Although this locomotor circuitry is not fully understood, most spinal cord injury (SCI) occurs above it so it may be harnessed for improving motor function after SCI. The SCI-induced loss of voluntary control is likely due to both a reduction in descending controls and spinal plasticity, involving alterations in network excitability and/or defects in neural processing. Locomotor rhythm generating interneurons (INs) are an obvious entry point for studying SCI and treatment. However, there is little information regarding the effects of SCI on these neurons or their connections, both afferent and efferent. Elucidating the alterations to these funtional circuits, following SCI, will enhance our understanding of the beneficial as well as the maladaptive plasticity that occurs in the spinal cord. The resultant insights will provide pathways for a more targeted and refined approach to treatment. The main objective of the present study is to identify targets for enhancing locomotor function, focusing on Shox2 rhythm generating INs. Complete thoracic spinal transections were performed on adult Shox2cre;Rosa26tdTomato mice. Whole cell patch clamp recordings targeted Shox2 INs in spinal slices from uninjured and chronic SCI adult mice. Shox2 INs displayed a wide reportoire of firing properties which can be linked to underlying voltage-gated currents. There were no major differences in excitability properties between control and SCI Shox2 INs. A fraction of Shox2 INs from both uninjured and SCI mice showed plateau properties, persistant inward currents, and displayed spontaneous bursting properties which were enhanced in the presence of serotonin. Additionally, a subset of Shox2 INs displayed monosynaptic excitatory postsynaptic potentials in response to low threshold afferent fiber stimulation. Current focus is on how the fidelity of afferent-evoked responses in Shox2 INs changes post-injury. In summary, the intrinsic properties of adult Shox2 INs are relatively resilient to SCI-induced plasticity, leaving them as robust targets for post-injury therapies.

Disclosures: **S. Bibu:** None. **N. Ha:** None. **L. Yao:** None. **K.J. Dougherty:** None.

Poster

627. Spinal Cord Injury: Rehabilitation

Location: Halls B-H

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Program#/Poster#: 627.29/AAA1

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NICHD R01 HD081274

Wings for Life-US-026/14

Title: Endpoint variability during overground walking in persons with chronic incomplete spinal cord injury

Authors: *W. SOHN, A. Q. TAN, D. PETERS, Y. THIBAUDIER, R. TRUMBOWER; Rehabil. Med. and Physical Therapy, Emory Univ., Atlanta, GA

Abstract: Incomplete spinal cord injury (iSCI) disrupts spinal neural pathways that contribute to sensorimotor deficits in the lower limb. In persons with iSCI, the resulting impairments decrease functional walking ability as their foot trajectory is often irregular, jerky, and variable from step-to-step. Gait variability is a relevant marker of gait instability (e.g., fall risk) and cortical control given the inverse association between variability and stability during walking. Therefore, we propose that evaluating the variability in endpoint foot kinematics during overground walking may elucidate the relationship between endpoint variability and walking recovery in persons with chronic iSCI.

The purpose of this study was to evaluate the effect of cadence and auditory cues on endpoint variability in persons with chronic iSCI during overground walking as compared to age-matched able-bodied (AB) persons with and without assistive device (AD). We hypothesized that persons with iSCI will exhibit greater variability in step-to-step endpoint foot-trajectory, as well as, greater endpoint variability with decreasing cadence as compared to AB during overground walking. To test our hypotheses, chronic iSCI participants and their age-matched AB controls were asked to walk at self-selected (SS), cadence-matched self-selected (SSmet), and fast cadence (Fmet) using an audible metronome. AB participants also walked at iSCI-matched cadences with AD. We measured kinematics of overground walking using motion analyses. We quantified variability of endpoint foot-trajectory during overground walking by computing the mean spatial density of metatarsophalangeal (MTP) marker in the sagittal plane during swing phase of gait.

Consistent with our hypotheses, we observed that the spatial path of the MTP in persons with iSCI was more variable than matched AB subjects at all cadences (SS, SSmet, Fmet). We also found that endpoint variability decreased at faster cadences in iSCI participants. However, the use of a metronome reduced variability, suggesting that walking variability can be regulated by walking cadence and auditory cues in persons with iSCI. Furthermore, in AB participants we

observed that walking with AD resulted in greater endpoint variability compared to no AD. These preliminary results suggest that training persons with iSCI subjects to walk at faster cadences or with auditory cues may improve gait stability by reducing endpoint gait variability and subsequently lead to improved walking ability after iSCI.

Disclosures: W. Sohn: None. **A.Q. Tan:** None. **D. Peters:** None. **Y. Thibaudier:** None. **R. Trumbower:** None.

Poster

627. Spinal Cord Injury: Rehabilitation

Location: Halls B-H

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Program#/Poster#: 627.30/AAA2

Topic: E.09. Spinal Cord Injury and Plasticity

Support: The Grainger Foundation

NIH R21 NS087320

Title: Topographic anatomy and skeletopy of white swine spine and spinal cord: establishing a targeting atlas for spinal cord neuromodulation in a large animal model.

Authors: *A. A. MENDEZ, J. CALVERT, R. ISLAM, P. J. GRAHN, K. E. BENNET, K. H. LEE, I. A. LAVROV;
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Abstract: Background: Electrical stimulation of the spinal cord has emerged as a possible therapeutic to restore volitional motor control. However, the mechanisms by which stimulation restores function are not well-understood. To this end, we previously established a large animal swine model of spinal cord stimulation to allow development of stimulation technologies that can be rapidly translated to the clinic. However, there still remains lack of information on the variability of spine and spinal cord anatomy for this model. Additionally, to date, no correlation has been established for the relative locations of anatomical structures for successful implementation of this large animal model for spinal cord neuromodulation.
Methods: Adult white swine were used for this work. MRI and CT scans were performed on live, anesthetized animals. Image fusion was performed manually using anatomical landmarks within both CT and MR images. Next, anatomical measurements were performed as follows: (1) landmarks were established along the facet joints, transverse processes, anterior and posterior portions of the vertebral laminas, as well as dorsal spinal cord rootlets, (2) distances between these landmarks were measured, and (3) dimensions were arithmetically compared and averaged

across subjects. To reduce variability, three operators conducted anatomical measurements of vertebras, dorsal roots, rootlets and spinal cord diameter at each spinal level. Finally, histological sections in transverse plane of the spinal cord were carried out and were correlated to the appropriate spinal segment.
Results: Spine and spinal cord anatomy varied across animals. Ratios for vertebral bone size, segment length and distances within spine and spinal cord landmarks were established for appropriate navigation within the pig lumbar spine when compared to empirical navigation. Observed correlations between vertebra length, spinal segment length, and rootlets entry, allows location of spinal cord segments based on bone landmarks. Correlation between histological data and imaging scans with anatomical landmarks and dimensions provides a single high-resolution coordinate system across tested animals.
Conclusions: This work represents our first attempt to establish precise anatomy of swine spine and spinal cord that will allow target selection based on anatomical structures and will provide precise stereotactic delivery of electrodes for targeted electrical investigation of spinal structures.

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Poster

628. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 628.01/AAA3

Topic: E.10. Motor Neurons and Muscle

Support: DARPA

Title: Layer-specific dynamics of sensorimotor cortex during motor learning

Authors: *P. GAO;
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Abstract: Recent studies have shown that motor learning is accompanied by enhancement of synaptic strength and synaptogenesis in the sensorimotor cortex. Functional reorganization of local circuit activity in primary somatosensory (S1) and primary motor (M1) cortices is believed to support changes associated with motor learning. While characterization of functional changes at distinct cortical layers (e.g. layers II/III and V) is focus of extensive studies, an investigation that links these changes with synaptic molecular machinery has been elusive. In this study, I investigated the time-dependent relationship between the learning and consolidation of a motor task and the expression of protein kinase M zeta (PKMζ) and postsynaptic density protein 95

(PSD-95), two molecular factors key for synaptic plasticity and remodeling, in layers II/III and V of the sensorimotor cortex.
My results showed that PKMζ expression level increased significantly on layer II/III of S1 and layers II/III and layer V of M1 during late stages of a skilled reaching task and sustained to be higher with extended training. In contrast, PSD-95 showed an increased level of clustering size in M1 layer V during the early stages of learning that was not sustained with extended training. My studies suggest that the increase of PKMζ may participate in the long-term storage of well-learned motor skills, whereas the PSD-95 dependent structural modification of synapses may mainly contribute to the acquisition of new motor skills.

Disclosures: P. Gao: None.

Poster

628. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 628.02/AAA4

Topic: E.10. Motor Neurons and Muscle

Title: Direct synaptic input to spinal neurons from corticospinal and putative Ia axons during development

Authors: *S. FUKUDA[1], H. MAEDA[1], T. OHNO[1], H. KAMEDA[1], N. MURABE[1], N. ISOO[1], H. MIZUKAMI[2], K. OZAWA[2,3], M. SAKURAI[1];
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Abstract: Spinal motoneurons (MNs) received direct inputs from Ia fibers, however, not from corticospinal (CS) axons in adult rodents. In this study, we succeed in making transverse slice of spinal cord in good health from 2nd to 4th weeks rodents and found the monosynaptic connections from putative Ia afferent and CS axons to MN by the stimulation of the dorsal column. Using cervical cord slices prepared from rodent 2nd weeks after birth, a stimulating electrode was placed in the ventralmost part or in the middle part of the dorsal column where the CS tract (CST) or putative Ia afferents are located (Yoshida et al. 2006) respectively. Whole cell recordings were made from two groups of MNs retrogradely labelled with fluorescent cholera toxin B subunit injected into forelimb muscles or pectralis major muscle. When we recorded responses with fixed latencies and same time course in ACSF containing high concentrations of divalent cations (7 mM Ca2+, 3 mM Mg2+), we judged those responses to be monosynaptic EPSCs. To more selectively stimulate CS axons we employed optogenetic stimulation by

injecting an adeno-associated virus vector encoding channelrhodopsin-2 (ChR2) into the sensorimotor cortex on P0 animals. After recording we observed close contacts between CS axons and MNs with a confocal microscope. Monosynaptic EPSCs were recorded from forelimb MNs by stimulation of the CST or the middle part of the dorsal column. The latency of putative Ia afferents-evoked monosynaptic EPSCs was 2.7 ± 0.2 ms, which is in good agreement with previous reports. This latency was significantly shorter (about half) than the CST-evoked monosynaptic EPSCs (4.9 ± 0.2 ms). The amplitude of Ia-evoked EPSCs was larger than CST-evoked EPSCs. These Ia-induced EPSCs showed significantly clearer paired-pulse depression than CST-induced EPSCs. Thus the electrode placed in middle part of the dorsal column stimulated Ia afferent axons. Any monosynaptic EPSCs were not recorded from pectralis major MNs by stimulation of the CST, but EPSC frequencies increased by repetitive (10 ms interval, 10 times) stimulation in normal ACSF. Pectralis major MNs received direct input from Ia afferent fibers, however, received only polysynaptic inputs from CS axons. Optogenetic stimulation evoked monosynaptic EPSCs in forelimb MNs. We showed close contacts between these forelimb MNs and CS axons labelled with the ChR2 (9.4 ± 4.2 per MN). Almost half of these contacts (55.3%) colocalized with PSD-95 in their partner dendrites. It is intriguing that these results are analogous to the connection pattern seen in adult primates. We found the connection with putative Ia afferent and spinal neuron in rodent > 4th weeks after birth.

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Poster

628. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 628.03/AAA5

Topic: E.10. Motor Neurons and Muscle

Support: Lundbeck Foundation Project Grant R140-2013-13648

Title: The effects of aging on the axon initial segment and electrical properties of spinal motoneurones in the C57BL/6J mouse.

Authors: S. GOLTASH, M. H. JAKOBSEN, J. LEHNHOFF, K. P. DIMINTIYANOVA, L. GROENDAHL, *C. F. MEEHAN;
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Abstract: Our previous investigations in a mouse model of Amyotrophic Lateral Sclerosis (ALS) found differences related to action potentials and axon initial segments (AIS) at symptom

onset (Jørgensen et al 2015 SFN abstract). How mouse motoneurones age normally however, is unknown. Therefore, we conducted a series of experiments in male C57BL/6J mice at 100, 300-400 and 600-750 days old. Rotorod scores significantly declined with age ($P<0.0001$, n= 41 mice), confirming a decline in motor function. Intracellular recording was then performed in 18 mice (6 at 100 days old, 6 at 300-400 days old and 6 at 600-750 days old). Somatic action potentials had significantly lower amplitudes in 600-750 day old mice compared to 100 day old mice ($P<0.0001$). Consistent with this, we observed a significantly reduced rate of rise of somatic action potentials in 600-750 day old mice ($P<0.005$). The rate of fall was also significantly slower in 600-750 day old mice compared to 100 day old mice ($P<0.005$). Consequently, the action potential width at 2/3 amplitude was significantly wider in 600-750 day old mice than both the 100 and 300-400 day old mice. The rate of rise of the IS component of action potentials was not significantly different at any age. Post-spike after-hyperpolarization (AHP) amplitudes were significantly larger at 300-400 days compared to 100 days ($P<0.05$) but not significantly so at 600-750 days. Compared to 100 day old mice, AHP durations were significantly shorter at 300-400 days old ($P<0.05$) but returned to the 100 day old levels at 600-750 days old. These changes appeared not to influence the current-frequency slopes which were not significantly different between groups. Immunohistochemistry was performed on 6 mice at 300-400 days old and 7 at 600-750 days to label AISs and motoneurones with antibodies against Ankyrin G and ChAT respectively. AISs were slightly shorter (approx. 4 %, $P<0.05$) and wider (12%, $P<0.0001$) in the aged mice. In four 600-750 day old mice the nodes of Ranvier of motor axons in the ventral roots were labeled with antibodies against KV1.2, Caspr and voltage-gated sodium channels. In these mice a clear breakdown of the juxtaparanode-paranodal boundary was evident compared with 4 younger mice. Conclusions: While the changes in AHPs mirror that which has been observed in humans with ALS and our recordings in mouse ALS models, the changes related to amplitude/rate of rise of action potentials, and structural changes at the AIS however are directly opposite to what we have observed in symptomatic ALS mice. Our results therefore suggest that changes related to sodium channels that we previously observed at disease onset in ALS mice do not represent a normal or accelerated aging.

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Poster

628. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 628.04/AAA6

Topic: E.10. Motor Neurons and Muscle

Support: Thomas Hartman Center for Parkinson's Research at Stony Brook University

SUNY Brain Network of Excellence

Title: Acute intermittent hypoxia reduces rate-dependent depression of the H-reflex

Authors: *N. P. PHAGU[1], M. CATEGE[1], I. C. SOLOMON[2], W. F. COLLINS, III[1];
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Abstract: Acute intermittent hypoxia (AIH) produces a robust increase in inspiratory motor output that continues after cessation of the hypoxia. Although referred to as respiratory long-term facilitation (LTF), this AIH-induced neural plasticity is not limited to respiratory motor outputs and may have widespread effects on somatic motor systems. The aim of the present study was to assess the effect of AIH on the monosynaptic H-reflex, an electrophysiological correlate of the myotactic stretch reflex, with particular focus on possible changes in H-reflex rate-dependent depression (RDD). Experiments were conducted on urethane-anesthetized (1.4 g/kg), spontaneously breathing adult female Sprague Dawley rats (250-300g). The left hind limb was secured with the ankle in a flexed position and platinum needle electrodes were inserted percutaneously along the medial aspect of the distal Achilles tendon (2-3 mm separation) for stimulation of the posterior branch of the tibial nerve. H-reflex EMG responses were recorded using fine stainless steel wires inserted into the dorsal metatarsal region (active electrode) and the tip of the fifth digit (reference electrode) of the ipsilateral hind paw. To elicit the H-reflex, the tibial nerve was stimulated throughout the experiment using trains of 15 stimuli (0.2 ms pulse duration; 2-3x H-reflex threshold; 10 Hz) delivered every 50 seconds (0.02 Hz). H-reflex RDD was estimated within each stimulus train by calculating the ratio of H-reflex amplitudes (15th response (10 Hz) / 1st response (0.02 Hz)). Diaphragm EMG activity was simultaneously acquired to assess AIH-induced respiratory LTF. Following at least 30 minutes of baseline recording, rats were exposed to a single bout AIH protocol consisting of three five-minute episodes of hypoxia (10% O_2; 90% N_2) separated by five-minute exposures to room air after which data acquisition continued for at least 90 minutes. Prior to AIH, rats exhibited H-reflex RDD of 30 - 70% (i.e., amplitude ratio 0.7 - 0.3, respectively). Following AIH, up to a 30% reduction in H-reflex RDD was detected, and this reduction persisted for up to 90 minutes. The reduced H-reflex RDD following AIH was due to less H-reflex amplitude depression at 10Hz, and no change in H-reflex amplitude at 0.02 Hz was observed. These results indicate that AIH exposure can enhance active monosynaptic spinal segmental reflexes and thereby contribute to AIH-induced motor plasticity.

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Poster

628. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

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Program#/Poster#: 628.05/AAA7

Topic: E.10. Motor Neurons and Muscle

Title: Neurosteroid alfaxalone fails to alteration excitatory synaptic transmission to rat hypoglossal motor neurons

Authors: *P. P. THAKRE, M. C. BELLINGHAM;
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Abstract: Alfaxalone is widely used in veterinary anaesthesia; however, neuromotor excitation during anaesthesia limits its use in laboratory rodents. Prior work has shown that alfaxalone suppresses inhibitory synaptic transmission to hypoglossal motoneurons (HMNs) via activation of cannabinoid receptor-1 (CB_R-1) and transient receptor potential (TRP) channels. However, the effect of alfaxalone on excitatory synaptic transmission has not been established. We evaluated effects of alfaxalone on excitatory neurotransmission to HMNs and the presence of TRPM4 channels and CB_R-1 receptors by immunohistochemical localization. Whole-cell patch-clamp recordings were made from HMNs in 300μm-thick transverse brainstem slices from 7-14 days-old Wistar rats after sodium pentobarbitone anaesthesia. Spontaneous excitatory postsynaptic currents (EPSCs) were recorded as inward currents at holding potential of -60mV using CsCl-based internal solution and in the presence of strychnine-HCl (20μM). Alfaxalone was bath applied at 3μM concentration. Alfaxalone failed to significantly alter the spontaneous EPSC frequency, amplitude, half-width and rise-time. Immunohistochemical studies showed that both TRPM4 channel and CB_R-1 receptor proteins are widely expressed on HMNs, confirming the modulation of inhibitory transmission to HMNs. Our results suggest that alfaxalone produces hyperactivity of motor neurons by reducing inhibitory but not excitatory transmission. The failure of alfaxalone to modulate excitatory neurotransmission and the presence of TRPM4 and CB_R-1 proteins on HMNs indicate that alfaxalone-induced hyperactivity and hence, neuromotor excitation during anaesthesia, is primarily governed by its effect on inhibitory transmission, which can possibly be reduced by TRPM4 and CB_R-1 antagonists.

Disclosures: P.P. Thakre: None. **M.C. Bellingham:** None.

Poster

628. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

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Title: The modulatory effects of caffeine on the intrinsic properties of spinal lateral motoneurons: evidence for its dependence on adenosine A1-dopamine D1 receptor heteromers

Authors: *M. S. RIVERA OLIVER[1], Y. ALVAREZ-BAGNAROL[2], C. AYALA-SANTIAGO[4], E. MORENO[5], G. SEALE[3], L. PEREZ-PORTOCARRERO[2], O. ACEVEDO-ARUS[2], V. CASADO[5], S. FERRE[6], M. DIAZ-RIOS[2];
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Abstract: Caffeine is a known non-selective adenosine receptor antagonist whose actions include multiple sites within the brain, mostly by binding to adenosine A1 and A2A receptors (A1R and A2AR). It produces similar behavioral effects as other classical psychostimulants including increased motor activation, arousal, and having reinforcing effects related to indirect dopaminergic mechanisms that depend on heteromerization of A1R and A2AR receptors with dopamine D1 and D2 receptors (D1R and D2R) in the striatum, respectively. We recently performed extracellular recordings of ventral nerves from the lumbar cord of mice in the presence of serotonin (5-HT), NMDA and dopamine (DA), which are known to elicit locomotor activity in mammals, and showed that caffeine stimulates motor activity by blocking A1R, by potentiating the ability of dopamine to activate D1R. Also, perforated patch clamp recordings in lumbar cord slices showed that the effects of caffeine are specifically targeted at modulating the intrinsic membrane properties of spinal lateral motoneurons (MNs). We then investigated if these properties of caffeine depended on the existence of A1R-D1R heteromers within spinal MNs. Thus, we proceeded to assess the presence of A1R-D1R complexes within spinal lateral MNs

using electrophysiological and histological techniques. Basal concentrations of DA or NMDA perfusion in the presence of synaptic blockers of inhibitory and excitatory neurotransmission depolarized the membrane potential of most MNs reversibly. The addition of caffeine, in the presence of DA or NMDA, significantly depolarized the membrane potential, decreased the action potential after-hyperpolarization (AHP) and increased the firing frequency by 90% of the recorded MNs. Also, we were able to support our theory that caffeine exerts its neuromodulatory effects on the spinal lateral MNs via A1R-D1R heteromers when the perfusion of an A1R agonist before a D1R agonist completely blocked the modulatory effects of the D1R agonist. Finally, potential A1R-D1R heteromer in MNs were localized anatomically through immunohistochemistry and with the use of a proximity ligation assay using antibodies directed toward the A1R and the D1R. Our experiments suggest that the primary target for the neuromodulatory effects of caffeine in the lumbar region of the spinal cord are the lateral MNs and that the excitatory effects produced by caffeine onto this neuronal population is dependent on A1R-D1R heteromers.

Disclosures: **M.S. Rivera Oliver:** 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; Spanish Ministerio de Economía y Competitividad with European Regional Development Funds of the European Union [SAF2014-54840-R, NIDA intramural funds, NIH NIGMS 1P20GM103642, NSF DBI-1337284, NIH RISE 2R25GM061151-13. **Y. Alvarez-Bagnarol:** None. **C. Ayala-Santiago:** None. **E. Moreno:** None. **G. Seale:** None. **L. Perez-Portocarrero:** None. **O. Acevedo-Arus:** None. **V. Casado:** None. **S. Ferre:** None. **M. Diaz-Rios:** None.

Poster

628. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 628.07/AAA9

Topic: E.10. Motor Neurons and Muscle

Support: MDA Grant MDA236717

NIH Grant NS082573

Title: Synaptic strength and plasticity differ within the axial motor neuron pool in larval zebrafish

Authors: *W.-C. WANG, P. BREHM;
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Abstract: The ability to perform movements with variable intensities allows animals to interact properly with the surrounding environment. Movement intensities are governed by activation of different types of motor neurons and muscles. Although many studies have been done on the recruitment pattern of motor neurons and muscles when generating forces of different amplitudes and speeds, there is a lack of systematic examination of synaptic properties at the neuromuscular junction between different types of motor neurons and muscles. In larval zebrafish, axial motor neurons in the spinal cord are arranged with a dorsoventral gradient. Ventrally located, smaller and more excitable motor neurons are activated during slow swimming, while dorsally located, larger and less excitable motor neurons are only recruited at fast swimming or escape. Compared to recruitment patterns during swimming, less is known about how the activity pattern of these motor neurons is translated into muscle contraction. More specifically, is there also a systematic difference in synaptic strength and plasticity for outputs of different motor neurons?
We performed whole cell patch recordings on connected motor neuron and skeletal muscle cell pairs to examine end plate currents (EPCs) elicited by firing the motor neuron. We found that when firing the motor neuron at low frequencies, EPCs elicited by secondary motor neurons (SMNs) in either fast or slow muscles were less reliable, as well as smaller and more variable in amplitude compared with primary motor neuron (PMN) elicited EPCs in fast muscles. The larger SMN EPCs were sufficient to elicit an action potential in the fast muscle but smaller ones were subthreshold. In response to firing the SMN at high frequencies, a potentiation in EPC amplitude was seen during the high frequency firing and persisted for several seconds to a couple minutes after switching to low frequency firing. This short term plasticity was different from PMNs, where EPCs showed an initial depression in response to high frequency PMN firing. The differences in amplitude, variability, and plasticity of SMN and PMN outputs were further confirmed by using high-speed video recording to examine muscle contraction elicited by firing a single motor neuron. The kinetics of potentiation during high frequency motor neuron firing was modified by internal EGTA concentrations, suggesting a calcium dependent process. Our findings suggest a significant difference in motor output from motor neurons that were previously demonstrated to be involved in swimming at different intensities and reveal the importance of synaptic transmission properties at the neuromuscular junction in motor control.

Disclosures: W. Wang: None. **P. Brehm:** None.

Poster

628. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 628.08/AAA10

Topic: E.10. Motor Neurons and Muscle

Support: NICHD R15HD075207

ARL 67444-LS-H

Title: Non-reciprocal effects of KCNQ/Kv7 channel modulation on the excitability of spinal motoneurons in mouse neonates

Authors: *J. LOMBARDO, M. A. HARRINGTON;
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Abstract: Motoneurons (MNs) are referred to as the 'final common pathway' because they represent the final arbitrers of the neuro-motor transformation. They perform this task by integrating the information from incoming inputs into trains of action potentials, thus their passive and active membrane properties, also referred to as intrinsic excitability, constitute a central component of this neuro-motor transformation. The voltage threshold of spinal MNs in particular appears to be preferentially targeted in regulating their output. Indeed, it is modulated acutely during fictive locomotion but also persistently after physical training, operant conditioning, weight bearing, and spinalization. Recently, the inhibition of KCNQ/Kv7 channels has been shown to modulate the voltage threshold of hippocampal and cortical neurons. These channels form a slow non-inactivating K^+ current, also known as the M-current, which activates in the sub-threshold range of membrane potentials and regulates different aspects of neuronal excitability. These channels may be important modulators of excitability in MNs as well. A mutation in the KCNQ2 channel subunit has initially been implicated in myokymia, a form of involuntary, repetitive muscle contraction, resulting from the hyper-activity of the motor units. Electrophysiological recordings have confirmed the presence of a slow, non-inactivating, K^+ current sensitive to both muscarinic receptor-mediated inhibition and KCNQ/Kv7 channel blockers in turtle MNs, and immunohistochemical studies have also confirmed the presence of KCNQ2 channel subunits in the somata, axonal initial segment and nodes of Ranvier of rat MNs. Finally, recordings from rat sciatic nerves demonstrated that KCNQ/Kv7 channels underlie the slow K^+ current at nodes of Ranvier and affect axonal conduction velocity. Although these initial findings highlighted the importance of KCNQ/Kv7 channels in spinal MNs, the function that these channels play in the neuro-motor transformation, and in the regulation of MN voltage threshold is unclear. To increase our understanding of the function of KCNQ/Kv7 channels in regulating the intrinsic excitability of spinal MNs, we have investigated the impact of modulators of KCNQ/Kv7 channels in spinal MNs whole-cell patch-clamp recordings obtained from acute neonatal mouse spinal cord slices. Using a combination of electrophysiological recordings and computational modelling, we demonstrate that KCNQ/Kv7 channel modulators contribute to the alteration of the axosomatic spinal MN excitability, even though the up- and down-modulation of their activity has non-reciprocal effects.

Disclosures: J. Lombardo: None. **M.A. Harrington:** None.

Poster

628. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

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Topic: E.10. Motor Neurons and Muscle

Support: This work was supported by a grant to CABMC (Control of Animal Brain using MEMS Chip) project funded by the Defense Acquisition Program Administration (UD140069ID).

Title: The location of neurons innervating the triceps brachii muscle of the pigeon as revealed by the retrograde transporter of the cholera toxin B subunit

Authors: *J. PARK[1], J. CHO[1], T.-K. LEE[1], I. KIM[1], J. AHN[2], K. SEO[3], M.-H. WON[1]; [1]Kangwon Natl. Univ., Chuncheon, Korea, Republic of; [2]Hallym Univ., Chuncheon, Korea, Republic of; [3]Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Many researchers have investigated the anatomical organization of the motor neurons which control behavioral output in mammals using various techniques. However, the anatomical distribution of motor neurons in the avian brain is still not fully elucidated. The purpose of this study was to finely determine the localization of the neurons innervating the triceps brachii muscle in the pigeon. The study was carried out in a total of 6 pigeons. The cholera toxin B subunit (CTB) was employed as a retrograde tracer to determine the location of the neurons of the triceps brachii muscle in the telencephalon of the pigeon following intramuscular injection. The animals were sacrificed at 14 days after intramuscular injection with CTB. We found that labelled neurons with CTB were located contralaterally in the nucleus basalis of the frontal telencephalon. This study shows that CTB is easily taken up by nerve terminals which are located in the triceps brachii muscle of the pigeon and motor neurons in the contralateral nucleus basalis may be closely associated with the movement of triceps brachii muscle.

Disclosures: **J. Park:** None. **J. Cho:** None. **T. Lee:** None. **I. Kim:** None. **J. Ahn:** None. **K. Seo:** None. **M. Won:** None.

Poster

628. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 628.10/AAA12

Topic: E.10. Motor Neurons and Muscle

Title: A novel mutation in the VCP gene identified by analysis of sporadic amyotrophic lateral scrlerosis in Japan

Authors: *H. SAKAMOTO[1], M. HIRANO[1,2], S. UENO[1], C. ISONO[1], M. NISHIDA[1], K. SAIGOH[2], Y. NAKAMURA[1], S. KUSUNOKI[2];
[1]Dept. Neurology, Kindai Univ. Sakai Hosp., Sakai, Osaka, Japan; [2]Neurol., Kindai Univ. Fac. of Med., Osakasayama, Osaka, Japan

Abstract: Background: Most cases of amyotrophic lateral sclerosis (ALS) are sporadic, but 5% to 10% are familial (FALS). Recent reports demonstrated that more than 10% of patients with sporadic ALS (SALS) have mutations in genes causative for FALS, suggesting a genetic-based pathomechanism in such cases. Mutations in the *VCP* gene encoding valosin-containing protein (VCP) cause inclusion body myopathy with Paget's disease of the bone and frontotemporal dementia (IBMPFD) and ALS. A recent study from China failed to identify any mutations in this gene in 324 patients with SALS. Methods: We sequenced the *VCP* gene in 90 Japanese patients with SALS. We constructed plasmid vectors expressing GFP-fused wild-type and mutant VCP, transiently transfected them separately in neuroblastoma SH-SY5Y cells, and treated the cells with an oxidative stress inducer, l-buthionine sulfoximine (BSO). BSO is an inhibitor of synthesis of the free-radical scavenger glutathione. Results: We found a novel *VCP* mutation, p.Arg487His in a patient (patient 1). He had progressive bulbar signs and weakness with severe amyotrophy of all four limbs from the age of 61 yr. Deep tendon reflexes were diminished, but extensor plantar reflexes were present. He required a percutaneous endoscopic gastrostomy (PEG) tube and artificial ventilation through a tracheostomy tube at the age of 66. Higher functions and personality were apparently normal until the age of 69. His personality was mildly changed with decreased attention after the age of 70. He had an elder brother (patient 2) with dementia, which was later found to be frontotemporal dementia with parkinsonism. Parkinsonism is occasionally found in patients with *VCP* mutations. Unfortunately, he had already died, and no DNA or further detailed clinical information was available. Fibroblasts from patient 1 was susceptible to BSO-induced oxidative stress. Transient expression of the newly identified mutant as well as of known mutants also rendered SH-SY5Y neuroblastoma cells vulnerable to oxidative stress.
Discussion and Conclusion: The presence of the mutation in the Japanese population extends the geographic region for involvement of the *VCP* gene in sporadic ALS to East Asia. In addition,

our findings suggest the involvement of oxidative stress in the pathomechanism of VCP-related ALS.

Disclosures: **H. Sakamoto:** None. **M. Hirano:** None. **S. Ueno:** None. **C. Isono:** None. **M. Nishida:** None. **K. Saigoh:** None. **Y. Nakamura:** None. **S. Kusunoki:** None.

Poster

628. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls B-H

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Program#/Poster#: 628.11/AAA13

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH R01 NS026539

Title: Structural and functional correlates of variation in neuronal numbers in the locus coeruleus of zebrafish imaged with multiphoton and high-speed light sheet microscopy

Authors: M. J. FARRAR[1,2], *K. E. KOLKMAN[1], J. R. FETCHO[1];
[1]Cornell Univ., Ithaca, NY; [2]Messiah Col., Mechanicsburg, PA

Abstract: Norepinephrine (NE), a neuromodulator found throughout the CNS, acts by altering target neuron excitability and synaptic plasticity. Activity in the locus coeruleus (LC), a major source of NE in the brain, is associated with alertness, attention, and memory processing. Zebrafish are an ideal model organism in which to study the role of the LC in physiology and pathology due to the ease of creating transgenic models, transparency in the larval stage, and small size of the LC nucleus. Using a *Tg:ET-VMAT GFP* line confirmed by antibody staining, we examined zebrafish larvae at 5 days post fertilization using 2PEF microscopy. In agreement with previous studies, LC neuron cell counts ranged between 10 and 20 bilaterally, with a mean of 15. This variation–as much as 100%– is not unique to zebrafish.
To study the correlation between LC number and neurite projections, we performed single-cell electroporations in the *Tg:ET-VMAT GFP* line. Using 3-D Sholl analysis, we found no systematic differences in projection density proximal to the soma of individual neurons between fish with few and many LC neurons.
To explore whether neurite density in target areas distal to the soma is correlated with LC neuron count, we used CRISPR/Cas9-mediated knock-in to create a transgenic line (*NET:mCFP*) with a membrane-targeted CFP under the endogenous NE transporter (NET) promoter, allowing us to reveal all NE projections with high fidelity. We examined the optic tectum and the spinal cord since NE projections in these areas are predominantly from the LC. We found a positive correlation (n = 5 fish) between LC number and neurite density.

We investigated the activity of LC neurons by creating a transgenic line expressing nuclear localized GCamp6f (*NET: H2B-GCamp6f*). To achieve high-speed bilateral imaging of neuronal activity, we custom-built a high-speed light sheet microscope (based on Ahrens et al., 2013) that allowed for whole brain imaging at several brains a second. We imaged spontaneous activity in the LC neurons in intact larvae as well as ones with hindbrain spinal-projecting neurons backfilled with Calcium Green dextran to allow correlation of LC activity with activity of identified descending neuronal populations. Correlated activity was observed between LC neurons and cells associated with swimming behavior including putative MiV2 and 3 neurons as well as neurons in the nucleus of the MLF. The average activity of individual neurons in the LC was negatively correlated with LC neuron count (n = 9 fish). These findings suggest that fish with fewer LC neurons may maintain physiological NE levels at lower projection density by a compensatory increase in the activity of individual cells.

Disclosures: **M.J. Farrar:** None. **K.E. Kolkman:** None. **J.R. Fetcho:** None.

Poster

628. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

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Topic: E.04. Voluntary Movements

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Human Frontier Science Program

Japan Science and Technology Agency (PRESTO)

Title: Emergent properties in the cortex-wide dynamics during motor learning

Authors: ***H. MAKINO**, C. REN, A. KIM, N. KONDAPANENI, T. KOMIYAMA; UCSD, La Jolla, CA

Abstract: Learning involves brain-wide transformation of operation dynamics. However, our understandings of basic principles governing learning-dependent changes in macroscopic dynamics are poor. Here we chronically monitored cortex-wide activity of the mouse brain using wide-field calcium imaging while the mouse learned a motor task over two weeks. Distinct cortical modules were extracted from activity patterns using methods based on independent component analysis. Across these modules, we found that motor learning alters the speed, direction and robustness of the signal propagation. Over learning, activity of the cortical modules reached its peak faster and their sequential activity became temporally more compressed. Moreover, a second stream of activity flow emerged from anterior regions of the cortex during learning. Lastly, in activity space where each dimension corresponds to activity of individual cortical modules, trial-by-trial variability was reduced along behaviorally relevant axes after learning. These results reveal newly emergent properties of the macroscopic cortical dynamics during motor learning.

Disclosures: H. Makino: None. **C. Ren:** None. **A. Kim:** None. **N. Kondapaneni:** None. **T. Komiyama:** None.

Poster

628. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 628.13/AAA15

Topic: E.04. Voluntary Movements

Support: Ressler Family Foundation

Title: Axotomized neurons in recovery of premotor network function following ischemic stroke

Authors: *N. JACOBS[1], S. T. CARMICHAEL[2];
[1]Neurobio. & Behavior, Univ. of California, Irvine, Irvine, CA; [2]Neurol., UCLA, Los Angeles, CA

Abstract: Ischemic stroke causes major neurological deficits affecting millions of individuals worldwide each year. Neurorehabilitation is the primary therapeutic option for stroke recovery. During early recovery period (days to weeks) surviving peri-infarct tissue in cortex exhibits axonal sprouting and network reorganization. Axotomized neurons recovering from the recent loss of downstream targets could play a crucial role in guiding this network reorganization and re-establishing function with compensatory network architectures. Rapid intracellular injury signals such as early calcium waves and mitogen-activated protein kinase signalling effectors in axotomized cells may allow them to act as early responders. The potential for axotomized

neurons to act at the leading edge of stroke recovery was probed using viral expression of GCaMP6s for network dynamics in premotor cortex and turbo-RFP for retrograde labeling of pre-motor cortex cells with axonal projections into targeted stroke site at forelimb M1 cortex. A photo-thrombic (PT) stroke model was used. Network function was assessed at baseline, +1, +7, and +21 days post-stroke using two-photon microscopy of calcium transients in mice performing a simple walk-left walk-right cued motor task. Preparatory motor coding of neural trajectories (Li et al., 2016) and the relative information content of axotomized vs non-axotomized cells was assessed. Analysis of this data is ongoing.

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Poster

628. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls B-H

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Program#/Poster#: 628.14/AAA16

Topic: E.03. Basal Ganglia

Support: Dystonia Coallition/Dystonia Medical Research Foundation

Title: Head neural integrator is impaired in cervical dystonia

Authors: ***A. G. SHAIKH**[1], V. POPOV[2], V. SHABALOV[3], S. RAVEA[2], H. JINNAH[4], A. SEDOV[2];
[1]Neurol., Case Western Reserve, Moreland Hills, OH; [2]Russian Acad. of Sci., Moscow, Russian Federation; [3]Burdenko Scientific Res. Neurosurg. Institute,, Moscow, Russian Federation; [4]Emory Univ., Atlanta, GA

Abstract: The midbrain cephalomotor neurons in primate interstitial nucleus of Cajal convert pulse of neural discharge encoding head velocity to steady state response characterizing head position, hence serving as the neural integrator keeping the head steady in desired orientation. It is hypothesized that impairment in the neural integrator or its feedback might cause abnormal twisting, turning, and oscillations of the head characterizing a debilitating movement disorder called cervical dystonia. We tested this hypothesis by analyzing the single-unit activity of the eye only, head only, and eye-head neurons in midbrain Interstitial Nucleus of Cajal (INC) in response to eye and head movements during stereotaxic surgeries aimed to treat cervical dystonia. The control group, the eye only neurons, sustained their activity during eccentric vertical gaze holding. However, there was a rapid exponential decline in the discharge of the head only INC neurons, suggesting impairment in the neural integration of head movements. We then measured the activity of eye-head neurons, predicting that the deficit localized within the

neural integrator will cause decay in the responses of eye-head neurons during eyes as well as head movements. On the contrary, if the impairment is in the head movement feedback, then the eye-head combined neurons will sustain activity during gaze holding, but the activity of the same neuron will decline during head holding. The results suggested impairment in head movement feedback. Our experiments, therefore, supported the hypothesis that impaired feedback, for example, cerebellar or basal ganglia manifest in dystonia by affecting the function of head neural integrator.

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Poster

628. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

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Topic: E.03. Basal Ganglia

Support: Universidade Federal do ABC

Comissão de Aperfeiçoamento de Pessoal do Nível Superior (CAPES)

Fundação de Amparo a Pesquisa de São Paulo (FAPESP)

Title: Catalepsy-induced typical and atypical antipsychotics is regulated by L-NOARG and Fos B expression

Authors: *J. C. SILVA[1], S. C. G. PRIETO[1], E. A. DEL BEL[2], M. B. ECHEVERRY[1]; [1]Univ. Federal Do ABC, Sao Bernardo Do Campo, Brazil; [2]MEF-Physiology, Univ. de São Paulo, Ribeirão Preto, Brazil

Abstract: *Introduction:* The Nitric Oxide (NO) is an atypical neurotransmitter synthesized from L-arginine by the nitric oxide synthase (NOS). The NO has been implicated in various neural process like synaptic plasticity, on modulating neurotransmitters, neurotoxicity and some gene expression, like *fos* family. Furthermore, the systemic administration of NOS inhibitors has shown similar motor effects to antipsychotics treatment. Therefore, the goal of this study was to investigate if the tolerance to the cataleptic effect induce by the NOS inhibitor is related to the FosB protein modulation. *Methods:* C57BL male mice (n = 5-8/group) The animals received L-NOARG 15 mg/Kg on 1, 3 and 5 day 30 min before to haloperidol 1 mg/Kg, clozapine 20 mg/Kg or olanzapine 15 mg/Kg administration. They were then challenged once per day for 5 days with antipsychotics or vehicle. *Behavioral test*: Catalepsy test was evaluated 120 min after

of antipsychotic administration. *Immunohistochemistry*: Was used the primary antibody FosB (1:1000 sc-7203, Santa Cruz Biotechnology). The striatum was divided into 4 quadrants for the analyze Dorsolateral (DL), Dorsomedial (DM), Ventrolateral (VL) and nuclei accumbens (NAc). The NAc quadrant was subdivide in Core and Shell region. *Statistical analyses*: The data were analyzed with ANOVA one-way or repetitive measures followed by Duncan's post hoc test. *Results: Subchronic treatment with* both, antipsychotic and NOS inhibitor showed effect in the treatment, haloperidol [$F_{(3,18)}$= 11.700; P = 0.002], olanzapine [$F_{(3,18)}$= 7.160; P = 0.009] and clozapine group [$F_{(3,18)}$= 25.146; P < 0.001], with decreased cataleptic effect on last day in atypical antipsychotics groups (P < 0.05). The olanzapine group presented an increase in FosB protein expression in the shell region [$F_{(4,11)}$= 8.919; P = 0.003]. However, when was given in combination with NOS inhibitor increased FosB protein expression in all striatum (P < 0.05). On the other hand, with clozapine treatment was observed decreased expression in the dorso-lateral [$F_{(3,11)}$= 94.420; P < 0.001] and dorso-medial striatum [$F_{(3,11)}$= 32.931; P < 0.001]. But, subchronic treatment of clozapine with NOS inhibitor decreased the expression of FosB protein in all striatum (P < 0.05). *Conclusions:* The antipsychotics induced extrapyramidal symptoms regulating intracellular mechanism as a FosB protein expression in the striatal regions. In addition, a co-treatment with NOS inhibitor decreased the cataleptic effect induced by olanzapine or clozapine. Therefore, the Nitric Oxide could be an important regulator in the motor alterations produced by neuroleptics.

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Poster

628. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

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Topic: E.04. Voluntary Movements

Support: NSF1257923

NIH F31NS079036

MRC Senior non-Clinical Fellowship

NIH R01NS060921

Title: Low-dimensional population dynamics converge to a cyclical attractor over the course of a motor program

Authors: *A. BRUNO[1], M. D. HUMPHRIES[2], W. N. FROST[3];
[1]Univ. California San Diego, San Diego, CA; [2]Fac. of Life Sci., Univ. of Manchester, Manchester, United Kingdom; [3]Cell Biol. & Anat., Rosalind Franklin Univ. Of Med. and Sci., North Chicago, IL

Abstract: Neural control of motor behavior arises from the mass action of neuron populations. While individual neuron activity can correlate with specific aspects of movement, a parsimonious theory of motor control may require unravelling the underlying system embodied by a population. For example, rotational dynamics during movement initiation have been observed in neural populations in motor cortex of nonhuman primates. Similarly, theoretical accounts suggest neural populations generating repetitive ongoing movements embody cyclical attractors. To determine the extent to which low-dimensional rotational dynamics persist in a less constrained, ongoing motor program, we investigated the dynamical structure of the neural population implementing the locomotion motor program in the pedal ganglion of Aplysia californica. We obtained multi-neuron datasets of the rhythmic motor program using large-scale optical recording with a fast voltage sensitive dye. We discovered that the sustained low-dimensional, rotational dynamics of the locomotion motor program are directly implemented by a cyclical attractor network.
We found that the neural population in the pedal ganglion met the necessary and sufficient conditions for a cyclical attractor. Evoking the locomotion program caused population activity to rapidly settle into a low-dimensional, slowly decaying periodic orbit. Repeatedly evoking the program within each animal caused the population activity to converge on the same low-dimensional manifold. Periodic dynamics were highly consistent across animals, indicating that a common underlying dynamical system generated the heterogeneous spike patterns. Spontaneous perturbations of the population dynamics predominantly returned to the attractor. Finally, to show that the cyclical attractor manifold is the motor program, we were able to decode specific firing of the neck-projecting motor-neurons directly from the low-dimensional dynamics. Collectively, our results support the hypothesis that repetitive movement is directly generated by the neural implementation of a cyclical attractor. Inherent in these findings is the notion that population activity, and not specifics of single neuron firing, are key to motor control. Such discovery of the overlying principles governing the dynamics in neural systems may mark the beginning of a more global understanding of brain function.

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Poster

628. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

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CONACYT Ph.D. scholarship No. 369794

Title: Decreased volume of gray matter in different regions and their correlation with motor impairment in Huntington's disease by magnetic resonance imaging

Authors: *A. CAMPOS-ROMO[1,2], V. GÁLVEZ[2,3], G. RAMÍREZ-GARCÍA[2], J. FERNANDEZ-RUIZ[4];
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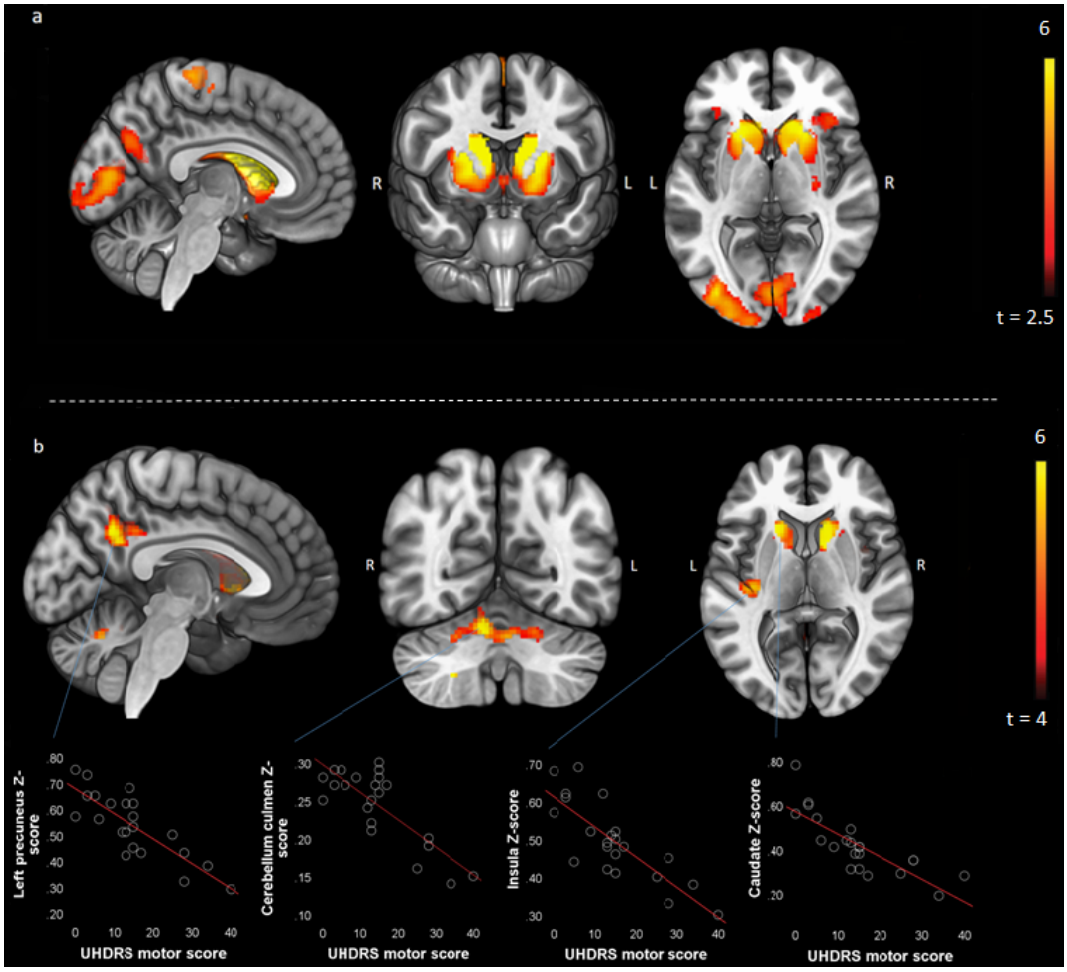
Abstract: Introduction: The striatum, a fundamental component of the basal ganglia is the main structure compromised in Huntington's disease (HD). However, it is still unknown if degeneration in other brain regions could also contribute to the major motor signs in HD patients at the early stages of the disease. **Objective:** Analyze, using voxel based morphometry (VBM), if degeneration in areas other than the striatum are linked to the major motor impairments in HD patients. **Method:** Twenty-two incipient molecular diagnosed HD patients, and 22 control subjects matched by sex and age, participated in this study. Patients were evaluated with the Unified Huntington's Disease Rating Scale (UHDRS). Structural T1 magnetic resonance images were acquired on a 3T scanner and used to obtain the volume of gray matter of each participant. **Results:** VBM analysis showed a significant negative correlation between the left insula, the culmen of the right cerebellum, the right cerebellar dentate, the right posterior cerebellum and bilateral cingulate gyrus with the motor UHDRS scale. In addition, as expected, regions such as caudate, putamen, sensorimotor and premotor cortices also correlated significantly with the motor scale (Figure). **Conclusion:** These results show that the motor alterations at the beginning of Huntington's disease does not exclusively correlate with basal ganglia degeneration, but thee motor deficits also correlate with damage in other brain regions involved in the control of movement, such as the cerebellum.



Disclosures: **A. Campos-Romo:** None. **V. Gálvez:** None. **G. Ramírez-García:** None. **J. Fernandez-Ruiz:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 629.01/AAA20

Topic: F.01. Neuroethology

Support: NSF IOS-145695

Title: Changes in the intrinsic physiology of premotor neurons as a result of auditory experience in the HVC of juvenile songbirds

Authors: *M. T. ROSS[1], D. FLORES[2], R. BERTRAM[2], F. JOHNSON[1], R. HYSON[1];
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Abstract: These experiments explore how auditory experience shapes the intrinsic physiology of premotor neurons during the developmental process of vocal learning in zebra finches. The learned vocalizations of songbirds are acquired over sensory and sensorimotor stages during development and require exposure to a tutor's song pattern. The cortical premotor nucleus HVC (not an acronym) is critical to the encoding and production of learned vocalizations in that proper functioning of HVC is required for the acquisition of song during sensory learning and for the proper timing of the adult vocal pattern (Roberts et al. 2012, Long et al. 2010). It is known that the HVC undergoes network level physiological changes during development (Day et al. 2013) and that learning drives changes in synaptic turnover, density, and morphology (Roberts et al. 2010, Peng et al. 2012), however it is unknown how the physiology of individual HVC neurons changes during learning and development. We have previously developed and tested kinetic models of adult HVC neurons and determined the role of several key ion channels in shaping their intrinsic physiology (Daou et al. 2013). This project explores how the intrinsic physiology of HVC neurons change over sensory and sensorimotor stages of learning in juvenile finches and explores the role that auditory experience has in shaping their intrinsic physiology. Using patch clamp electrophysiology, we recorded from distinct classes of HVC neurons across developmental learning stages and characterized the changes in their physiology. The results show changes in specific firing properties across stages of learning, such as a reduced rectifying response to hyperpolarizing currents in HVC_X projection neurons in juveniles compared to adults. We then developed biomathematical models in order to better characterize the neurons' physiology and predict changes in specific ion channel expression patterns. To explore whether learning was driving these changes in physiology, we performed patch clamp electrophysiology in the HVC of juvenile finches in a variety of tutor exposure conditions and compared their physiology to those who had normal tutor exposure. The results suggest that sensory learning does drive changes in the intrinsic physiology of HVC neurons. This would indicate that vocal motor encoding in songbirds not only requires changes in synaptic connectivity, but also changes in the intrinsic physiology of neurons through the alteration of ion channel expression patterns. These results give an improved understanding of the mechanisms of plasticity that the nervous system uses to encode new information or behaviors.

Disclosures: M.T. Ross: None. **D. Flores:** None. **R. Bertram:** None. **F. Johnson:** None. **R. Hyson:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

Location: Halls B-H

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Topic: F.01. Neuroethology

Support: NSF Grant IOS1456965

Title: Electrophysiological properties of neurons of the female zebra finch song nucleus and comparision with males

Authors: *D. FLORES[1], D. SHAUGHNESSY[2], F. JOHNSON[2], R. L. HYSON[2], R. BERTRAM[3];
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Abstract: Male zebra finches learn to sing by forming an auditory memory of song (listening to a tutor bird, typically its father) then using the memory as an internal reference to guide motor learning of a facsimile. In contrast, although female zebra finches form auditory memories of song, they don't sing. This striking behavioral difference is reflected in differences in the structure of the neural song system. Nucleus HVC (proper name), which plays a central role in both auditory and motor learning in males, is larger in males than in non-singing females. One possibility is that the smaller female HVC supports auditory, but not motor, learning. We performed an electrophysiological study of the properties of neurons from the female HVC, using the whole-cell patch technique to determine which types of ion channels contribute to the electrical behavior of the neurons. Differences in shape and duration of action potentials between female and male HVC projecting neurons suggest a distinction among sodium and potassium channels in these cells. This analysis was facilitated by the use of mathematical modeling, as we have done in a prior study of male HVC neurons. The behavior and characteristics of the projection neurons and interneurons of the female HVC were compared with those of male HVC neurons, again using modeling to facilitate the comparison. Finally, the dynamic clamp technique, which allows one to apply a model ionic conductance to a real cell, was used to determine how the addition or subtraction of an ionic conductance can make the electrical activity of the female neurons more like those of the male. Overall, we find consistent differences between male and female HVC neurons, both in the magnitude of channel conductances and in their kinetics.

Disclosures: D. Flores: None. **D. Shaughnessy:** None. **F. Johnson:** None. **R.L. Hyson:** None. **R. Bertram:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

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Program#/Poster#: 629.03/AAA22

Topic: F.01. Neuroethology

Support: NSF Grant IOS1456965

Title: A brainstem efference copy model for the control of singing in zebra finch

Authors: *D. GALVIS[1], R. L. HYSON[2], F. JOHNSON[2], R. BERTRAM[2];
[1]Mathematics Dept., [2]Program in Neurosci., Florida State Univ., Tallahassee, FL

Abstract: The cortical nucleus HVC (proper name) is largely responsible for the neural control of the learned song in the male zebra finch. Our lab has previously shown that bilateral medial ablations of the HVC produce variations in the sequencing of syllables in a bird's motif, while lateral ablations result in omitted syllables. These data suggest distinct roles for the medial and lateral portions of the HVC in the neural control of song. The centrality of the HVC in the timing of syllables was established earlier in studies showing that cooling the HVC uniformly stretched song syllable and gaps across multiple timescales (Long & Fee 2008). We present a computational neural network model that can account for the effects of medial and lateral ablations as well as the effects of temperature on patterned neural activity that controls song syllable timing and duration. A previous computational model assumed a role for brainstem feedback in the production of syllable sequencing while using synfire chains to control individual syllables (Gibb et al. 2009). We propose a modified brainstem efference copy model which explains medial and lateral ablations in terms of selective knockout of chains from within the medial HVC and lateral HVC representations of the network. "Medial ablation" within the model results in non-random atypical syllable sequences which can account for the transition patterns seen in the experimental data. "Lateral ablation" within the model results in syllable omissions and truncation of the song sequence. As with the experimental data, ablations within the model do not result in partial syllable production. This model unifies various theories regarding the role of HVC in the production of birdsong and is the first model that can explain the effects of targeted HVC ablations.

Disclosures: **D. Galvis:** None. **R.L. Hyson:** None. **F. Johnson:** None. **R. Bertram:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

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Topic: F.01. Neuroethology

Support: NSF Grant IOS-1456965

FSU Neuroscience Fellowship

Title: Brain connectivity not to blame for a sex difference in singing behavior of zebra finches

Authors: *D. W. SHAUGHNESSY[1], D. FLORES[2], L. MOORE[1], R. BERTRAM[2], W. WU[3], R. L. HYSON[1], F. JOHNSON[1];
[1]Psychology, [2]Mathematics, [3]Statistics, Florida State Univ., Tallahassee, FL

Abstract: Zebra finches (*Taeniopygia guttata)* exhibit a dramatic, binary sex difference in behavior. Males learn to produce a song, whereas female zebra finches do not. However, both sexes form auditory memories of songs, which are encoded in part by the cortical nucleus HVC (proper name). In males, HVC also directs the motor production of the learned song. For over 30 years, the absence of singing by female zebra finches has been attributed to the smaller size of female HVC (Nottebohm & Arnold 1976) and, in particular, the apparent anatomical disconnect between HVC and the vocal-motor nucleus RA (robust nucleus of the arcopallium; Konishi & Akutagawa 1985, Williams & Nottebohm 1985). We revisited HVC connectivity in female zebra finches, applying newly developed surgical and visualization techniques. Dye injections into female HVC showed male-like connectivity, with efferent outputs from HVC to RA and Area X (of the avian striatum), as well as afferent inputs from NIf (nucleus interfacialis) and Uva (nucleus uvaeformis). Although female HVC is smaller than in males, the sexes share similar extrinsic HVC connectivity. Thus, the absence of singing by females is not due to the lack of an efferent connection from HVC to the vocal-motor nucleus RA.

Disclosures: D.W. Shaughnessy: None. **D. Flores:** None. **L. Moore:** None. **R. Bertram:** None. **W. Wu:** None. **R.L. Hyson:** None. **F. Johnson:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

Location: Halls B-H

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Topic: F.01. Neuroethology

Support: The Parkinson's and Movement Disorder Foundation (PMDF)

The Michael J. Fox Foundation for Parkinson's Research/University of North Carolina Viral Vector Core

Title: Role of alpha-synuclein in Area X of adult male zebra finches: implications for acoustic variability in birdsong

Authors: *S. J. MUNGER, C. A. MEDINA, L. Y. SO, K. B. CHURCH, J. L. RITTER, J. E. MILLER;
Neurosci. Dept., Univ. of Arizona, Tucson, AZ

Abstract: Abnormal accumulation of alpha-synuclein is a key finding in the brains of individuals with Parkinson's Disease (PD). Rodent models have been developed to examine how alpha-synuclein protein expression is related to limb motor symptoms of PD through the use of transgenic mice and Adeno-Associated Viruses (AAV) targeted at dopaminergic pathways in rodents. Emerging evidence from these rodent models indicates that vocal changes precede the limb motor symptoms and could potentially be used for an earlier diagnosis of PD. These models, however, rely upon interpretation of vocal signals whose underlying neural circuits are not well-characterized. By contrast, the zebra finch (*Taeniopygia guttata)* songbird model has identified brain nuclei dedicated to vocal learning and on-going song maintenance. Experimental manipulations in these vocal control regions can be directly correlated with changes in song. To understand the role of alpha-synuclein in birdsong and to establish a model for the Parkinsonian vocal deficits, we characterized baseline levels of alpha-synuclein across non-singing and social-context dependent singing states, i.e. when an adult male sings alone (undirected song-UD) versus to a female (directed song-FD). Western blot analysis showed down-regulation in singing birds of alpha-synuclein protein in Area X, a song-dedicated sub-region of the basal ganglia. To establish the zebra finch as a genetic PD model, an alpha-synuclein overexpressing AAV was injected into Area X and compared to a Green Fluorescent Protein (GFP) control virus. Immunohistochemical analysis showed that unilateral injections of the alpha-synuclein AAV into an Area X hemisphere resulted in higher levels of the protein compared to the control virus-injected side. Bilateral injections into Area X were done with either alpha-synuclein or GFP control virus with song recorded pre and post-injection over three months. We hypothesized that alpha-synuclein overexpression would decrease the level of acoustic variability in the bird's UD and FD songs. Birds that received alpha-synuclein virus showed subtle changes in acoustic

features of song syllables. On-going work is investigating the impact of alpha-synuclein overexpression on dopaminergic pathways within Area X as they relate to the occurrence of vocal deficits in humans.

Disclosures: S.J. Munger: None. **C.A. Medina:** None. **L.Y. So:** None. **K.B. Church:** None. **J.L. Ritter:** None. **J.E. Miller:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 629.06/AAA25

Topic: F.01. Neuroethology

Title: Behavioral regulation of dopamine biomarkers in Area X of adult male zebra finch songbirds

Authors: *L. Y. SO, S. J. MUNGER, J. E. MILLER;
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Abstract: Dopamine (DA) is an important neuromodulator of motor control across vertebrate species, and under neuropathological conditions, the loss of DA in the basal ganglia leads to Parkinson's Disease (PD). Vocal deficits associated with PD include monotonous voice, decreased loudness, and articulatory problems; however, the link between the loss of DA and vocal modulation is not well-understood. In rodent and songbird models, 6-hydroxydopamine (6-OHDA), a neurotoxin that causes dopaminergic cell death, has been used to study the effects of DA loss on vocalizations. Zebra finch (*Taeniopygia guttata)* songbirds are vocal learners and have similar dopaminergic pathways as in the human brain making them an advantageous model to study the role of DA in vocalizations. The zebra finch songbird model has been used to characterize the role of DA in modulating neuronal firing patterns in Area X when the bird is singing by himself (undirected, UD) or to a female (directed, FD). In the current study, we investigated whether the vocal behavior of the zebra finch (non-singing vs. UD/FD) regulates the protein expression of different biomarkers associated with dopaminergic activity in Area X, including tyrosine hydroxylase (TH), an enzyme required for DA biosynthesis. Two hours following the non-singing (NS) or singing states, a key time-point used to examine song-related changes in gene expression, the brain was extracted and Area X tissue biopsied for Western blotting. We hypothesized that TH levels in Area X would be higher during singing versus NS as well as higher in FD than UD due to previous studies sampling DA metabolites and given the rewarding nature of FD courtship behavior. TH levels were differentially regulated in Area X across NS and singing states and may be regulated by the amount of song and social-context (UD

vs. FD). On-going experiments are investigating protein expression patterns in NS and singing states for DA receptors and other biomarkers in Area X. Future work will examine how 6-OHDA-induced DA depletion in Area X affects DA biomarkers in different vocally-driven behavioral states. The goal is to understand the neurobiological mechanisms whereby DA contributes to normal vocal behavior and the consequences of DA loss as noted in PD.

Disclosures: L.Y. So: None. **S.J. Munger:** None. **J.E. Miller:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

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Topic: F.01. Neuroethology

Support: Scientific & Technological Cooperation Programme Switzerland-Russia

Swiss National Science Foundation 31003A_127024

European Union ERC-2010 AdG 268911

Title: Formation of hierarchical network of vocalizations in songbird groups

Authors: *A. L. VYSSOTSKI[1], V. N. ANISIMOV[2], A. V. LATANOV[2], R. H. R. HAHNLOSER[1];
[1]Inst. of Neuroinformatics, Univ. of Zurich and ETH Zurich, Zurich, Switzerland; [2]Fac. of Biol., Moscow State Univ., Moscow, Russian Federation

Abstract: Vocalizations are an essential part of social interactions in birds. They have important biological functions in coordinating activity of group members. In the previous study (SfN 2013, 549.10) using back-attached ultra-miniature sound/acceleration logger (Anisimov et al., Nat. Meth. 2014) we recorded vocalizations in groups of freely moving laboratory-housed zebra finches and have found that communication networks in zebra finch groups have a stable hierarchical structure.
The networks formed and strengthen over the course of several days in newly created groups (from animals that never have been in contact with each other), where the strengthening was manifested in faster and more reliable call-call responses. Singing behaviors differed between individuals. Some animals tended to sing together whereas others avoided that tendency. Introduction of the female strongly decreased the number of songs produced by males and changed the hierarchy of their call interactions within the entire group. After female removal the original hierarchy recovered in most cases.

However, a sudden creation of a group from unfamiliar birds is probably a rare event in the wild where stable communities of flocking birds take place. Thus, we decided to verify the dynamics of vocal interactions in a group formed from familiar birds that we temporarily separated before recording sessions. We recorded vocalizations in a group formed by four adult brothers from the same clutch (216-220 days-post-hatch) and their father (1049 dph at the beginning of the group recording session). Animals were separated from each other about 150 days before recording, but were kept in cages together with other birds. Forty days before the communal recording session animals were placed in individual sound isolation chambers for song recording and habituation to the backpack weight.
After placing animals in the communal recording chamber their hierarchical network of vocalizations was established within the first 2.5 hours and was very robust during the following week. The most intensive vocal interactions were observed on the first day. Whereas only 4 from 12 unfamiliar birds demonstrated pairwise co-singing (33%), all siblings (n = 4) sang in stable pairs (100%). The father always lead vocalizations of the group in spite of the smallest number of calls produced. Presumably, an important factor contributing to fast formation of a hierarchical network of call interactions is familiarity of animals. And, we speculate that zebra finches do not forget their relatives even after long separation and that memorization and recognition might be facilitated by song similarity.

Disclosures: **A.L. Vyssotski:** None. **V.N. Anisimov:** None. **A.V. Latanov:** None. **R.H.R. hahnloser:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

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Topic: F.01. Neuroethology

Support: Hearing Health Foundation #23291

Title: HVC controls song timing by synchronizing the activation of multiple respiratory motor systems

Authors: *C. M. URBANO[1], J. M. MÉNDEZ[2], B. G. COOPER[1];
[1]Dept. of Psychology, Texas Christian Univ., Fort Worth, TX; [2]Dept. of Physics and Astronomy, Minnesota State Univ., Mankato, MN

Abstract: Birdsong, like human speech, is a vocal behavior that requires the formation of long-term auditory memory. Vocal reproduction of the memorized acoustic model requires that birds

precisely coordinate the activation of respiratory and vocal organ motor systems. In both juvenile and adult songbirds, ablation or inactivation of HVC (proper name) neural activity disrupts temporal features of song. Consistent with the role of respiration in controlling song tempo, we have previously demonstrated that HVC contributes to song respiratory production in adult male Bengalese finches (*Lonchura striata domestica*, Urbano & Cooper, 2015). Here we further explored these findings by recording subsyringeal air sac pressure and syringeal (avian vocal organ) electromyographic activity in singing birds prior to, during, and following recovery from HVC inactivation. Dialysis probes were implanted into either left or right HVC in adult male Bengalese finches (N=5), allowing for reversible suppression of neural activity (muscimol, 1.5 mg/mL). In all animals, mean air sac pressure and initial (10 ms) slope of song-related expiratory pulses (EPs) dropped significantly during HVC inactivation and recovered (Amplitude: $F(2,8) = 12.8$, $p = 0.003$; Slope: $F(2,8) = 15.3$, $p = 0.002$). Subsyringeal air pressure is driven by the combined activation of expiratory muscles (*m. obliquus externus abdominis*) and syringeal adductor muscles (*m. tracheobronchialis dorsalis*, dTB). To investigate the mechanism underlying the reduced air pressure amplitude and slope, the onset time of dTB activity (n=3) or expiratory muscle activation (n = 1) relative to EP onset was measured. During baseline song, dTB activity preceded song EP onset by 5-20 ms and expiratory muscle activation preceded EP onset by 10-15 ms. During right HVC inactivation, left (n=1) and right (n=2) dTB activity was delayed by ~10 ms in a subset of syllables with activation occurring atypically after EP onset. In contrast, we did not observe a similar temporal delay in activation of expiratory muscles, but did observe a decrease in activation amplitude. However, unilateral HVC inactivation did not affect air pressure amplitude, slope, or the timing of dTB activity during the production of learned contact calls. These results suggest that song degradation after HVC lesion or inactivation is linked to temporal changes in dTB activity and reduced activation of expiratory muscles. Therefore, HVC controls song timing by synchronizing diverse motor systems required to reproduce respiratory patterns that generate sequential, learned vocalizations.

Disclosures: **C.M. Urbano:** None. **J.M. Méndez:** None. **B.G. Cooper:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

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Topic: F.01. Neuroethology

Support: Incopix DECODE award

Title: Imaging neural representations of learned vocalizations in the basal ganglia

Authors: *J. SINGH ALVARADO[1], M. G. KEARNEY[1], R. MOONEY[2];
[1]Neurobio., Duke Univ., Durham, NC; [2]Neurobio., Duke, Durham, NC

Abstract: The basal ganglia (BG) plays a key role in coordination and execution of complex behaviors. Songbirds represent a tractable experimental platform for studying the mechanisms by which BG neuronal ensembles encode, drive, and modify behavior. Indeed, song learning and production is orchestrated by a specialized network of anatomically discrete brain nuclei including the BG homologue Area X. The dedicated nature of this system provides a rare opportunity in which BG population activity can be directly linked to a readily quantifiable behavior such as song. To investigate neural representations of song we performed deep brain calcium imaging of Area X neurons with a miniature head mounted 1 photon microscope. In initial experiments we used a nonspecific viral approach to pan-neuronally label all cell types in songbird BG with the genetically encoded calcium indicator, GCaMP6s. Calcium signals from single neurons revealed neural signatures of vocal initiation and termination, as well as stereotyped activity during song. Area X contains intermixed neuronal cell types including medium spiny neurons (MSNs), pallidal neurons (GPi- and GPe-like neurons). As MSNs are hypothesized to be a critical site of plasticity underlying learning, we sought to selectively monitor their activity during singing. We employed a CaMKII promoter-based strategy to virally label cells whose size is consistent with MSNs and confirmed that viral expression of the reporter construct co-localized with an MSN marker (DARPP-32). In vivo imaging of genetically identified MSNs during singing demonstrated distributed sequential activity throughout the song motif. Longitudinal measurements from identified BG neurons have the potential to describe the fundamental neural changes that enable the acquisition and maintenance of a complex vocal behavior.

Disclosures: J. Singh Alvarado: None. **M.G. Kearney:** None. **R. Mooney:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

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Program#/Poster#: 629.10/BBB3

Topic: F.01. Neuroethology

Support: HHMI

R01 MH055987

Title: Songbird basal ganglia enable context-dependent motor skill adaptation

Authors: *L. Y. TIAN, M. S. BRAINARD;
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Abstract: Adaptive behavior depends on the ability to flexibly organize individual actions, or gestures, into sequences. Crucial to the optimal performance of action sequences is the ability to vary how an individual gesture is executed, depending on which sequence it is embedded in (i.e. its sequential “context"). An example is coarticulation in speech, where a given gesture, or phoneme, can be executed with different articulator (e.g. lip) positions depending on its sequential context (e.g. what word it is in) in order to enable smooth transitions between the phonemes that make up each word. Here we investigate the neural substrates that underlie context-dependent modification of individual motor gestures in Bengalese finch song. Bengalese finch song consists of sequences of syllables that are variably ordered from rendition-to-rendition, such that the same syllable can be sung in different sequential contexts. We found that we could use aversive reinforcement to train birds to modify the same syllable differentially in different contexts. Moreover, the expression of such context-dependent modifications depended on the anterior forebrain pathway (AFP), a basal ganglia-pallial circuit important for song plasticity. Specifically, pharmacological inactivation of the output nucleus of the AFP blocked the full expression of modifications that were sequence-dependent (resulting from differential training in different contexts), but not modifications that were sequence-independent (resulting from the same training in all contexts). Our results indicate that the AFP integrates information about sequential context and learning signals in order to enable adaptive, context-dependent modifications of syllables. More broadly, we suggest that other forms of context-dependent motor adaptation, for which neural substrates are unclear, might similarly depend on basal ganglia circuits.

Disclosures: L.Y. Tian: None. **M.S. Brainard:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

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NINDS Grant NS087506

NINDS Grant NS092299

Title: Development of a representation of self-generated vocal behavior in songbird vocal motor cortex

Authors: *R. C. YUAN, S. W. BOTTJER;
USC, Los Angeles, CA

Abstract: Similar to speech acquisition in infants, vocal learning in juvenile zebra finches entails a process of sensorimotor integration in which auditory feedback of self-produced vocalizations guides refinement of variable immature vocal sounds into stereotyped adult patterns. In zebra finches, the output of this sensorimotor processing is conveyed to RA, a region of motor cortex that drives vocal output. Neurons in RA of adult zebra finches demonstrate greater response strength to playback of the bird's own song than to playback of conspecific songs. However, little is known about how the responsivity of RA neurons in juveniles engaged in sensorimotor learning compares to that in adults: do neurons in juvenile RA also demonstrate greater selectivity for the bird's own vocalizations? Alternatively, juvenile RA neurons may maintain broad responsivity to all possible motor outputs, thereby facilitating exploration of variable vocalizations during learning. We investigated this question by making extracellular recordings in RA of anesthetized juvenile (40-48 dph) and adult (> 90 dph) male zebra finches and testing the neural response to playback of each bird's own song (OWN) against playback of a mirror reverse of each bird's own song (REV), juvenile and adult conspecific songs (CON), and the adult tutor song. Single neurons in RA of juvenile birds were broadly responsive: 82% of recorded neurons demonstrated a significant increase in mean firing rate during playback of two or more song stimuli. Across all recordings, response strength to playback of each juvenile song (OWN, REV, juvenile CON) was greater than the response to the adult tutor song ($n = 41$ units; $p < .05$). However, response strength did not vary among playback of juvenile song stimuli. This pattern of results suggests that neurons in RA of juvenile birds prefer acoustic features inherent to immature juvenile vocalizations over adult vocal patterns, but do not prefer the bird's own song over other immature vocal sounds. In contrast to juveniles, neurons in RA of adults demonstrated greater response strength to playback of OWN compared to all other stimuli – including the acoustically similar tutor song ($n = 31$ units; $p < .05$). Moreover, the magnitude of selectivity for OWN over tutor song was greater in adults than in juveniles ($p < .01$). These results indicate that RA neurons respond to playback of various juvenile vocal sounds during early learning but develop a strong preference for the bird's own vocalizations by the end of sensorimotor development. This pattern suggests that selectivity of RA neurons may be shaped more by auditory feedback of self-produced sounds than by auditory experience with the tutor song.

Disclosures: R.C. Yuan: None. **S.W. Bottjer:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

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NIH grant DC002524

NIH grant NS079929

Title: Distributed mechanism of song timing generation

Authors: *K. HAMAGUCHI[1], M. TANAKA[2], R. MOONEY[2];
[1]Kyoto Univ., Kyoto, Japan; [2]Duke Univ., Durham, NC

Abstract: Timing control is a fundamental aspect of precise motor control. Birdsong is an elaborate and stereotyped vocal behavior controlled with millisecond precision. Various lines of evidence support the hypothesis that song premotor neurons located in a telencephalic nucleus (HVC) form a synaptic chain to generate song tempo. On the other hand, vocalization also depends on the brainstem circuits that involve the control of respiratory, expiratory rhythms and muscles in syrinx. How the forebrain and brainstem interact to produce temporally precise and reproducible behaviors is not well understood. Here we combine physiological, dynamical and computational methods to show that song tempo is the product of a distributed and recurrent synaptic network spanning the forebrain and brainstem. Using a miniature Peltier device, we found that focally manipulating the temperature of HVC exerted nearly identical effects on song tempo and activity propagation through a recurrent, distributed network that contains HVC as one of its elements. In contrast, cooling HVC exerted three to four fold greater effect on activity propagation locally within HVC than it did on song tempo. Moreover, focally manipulating temperature at a thalamic node in the recurrent network also altered song tempo, and these effects could not be attributed to thermal effects on HVC. Finally, intracellular recordings in singing birds reveal that different HVC premotor cells, which generate sparse and sequential action potential activity during singing, receive high frequency and synchronous patterns of depolarizing postsynaptic potentials, a synaptic feature that is inconsistent with simple local chain models studied so far. We confirmed that a simple distributed chain model can account for the temperature sensitivity and synchronous synaptic activity underlying the sparse sequential action potential activity. These findings indicate that precisely timed neural activity critical to the

regulation of song tempo arises from a distributed, recurrent network rather than through a localized mechanism.

Disclosures: K. Hamaguchi: None. **M. Tanaka:** None. **R. Mooney:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 629.13/DP09 (Dynamic Poster)

Topic: F.01. Neuroethology

Support: NWO Rubicon 825.15.007 to S.M.

NIH R01NS089679-01 to T.J.G.

Title: Sleep may promote maintenance of stable motor performance in songbirds

Authors: *S. MOORMAN, W. A. LIBERTI, III, L. N. PERKINS, B. H. PRICE, T. J. GARDNER;
Biol., Boston Univ., Boston, MA

Abstract: Zebra finch males sing a single, stereotyped song, hundreds of times per day, and this song is produced with remarkable precision for a bird's entire adult life. It is not known how a neural network can maintain this stable behavior on timescales of months or years. A premotor brain region called HVC (used as a proper name) is crucially important for song production: Lesions or perturbations of normal activity in HVC during song learning prevent the juvenile from learning to sing (Roberts, Gobes, et al., 2012), and during adulthood disturb song performance (Williams et al., 1992; Long & Fee, 2008; Wang et al., 2008; Basista et al., 2014). Cells in HVC that project to downstream brain regions (motor cortex, basal ganglia and a secondary auditory brain region) show extremely stereotyped activity patterns during singing: they burst sparsely, precisely aligned to an exact position in the song (Hahnloser et al., 2002; Markowitz, Liberti, et al., 2015). When the bird is awake and not singing, these cells are rarely active. However, it has been observed that spontaneous replay of sequences of neural activity occur in the song system throughout sleep (Dave & Margoliash, 2000; Chi et al., 2003; Hahnloser et al., 2006; Crandall et al., 2007; Shank & Margoliash, 2009; Moorman, Gobes, et al., 2015). We hypothesized that sleep might be important for the maintenance of behavioral stability of song. To study activity of HVC projection neurons during natural sleep, we used a genetically encoded calcium indicator in conjunction with head-fixed fluorescence microscopy. Here, we demonstrate that sleep-related activity of HVC projection neurons differs from daytime

singing. The statistical structure of spontaneous activity in sleep is more variable than during singing: the temporal correlations between cells are less stereotyped, and the duration distribution of sleep replay events is broad. We propose that noisy spontaneous activity in sleep might be integral to maintaining stable motor patterns on a long timescale, by promoting variability in the song motor program that serves as a substrate for adaptive plasticity during singing. In support of this, we observe that projection neurons in the song motor system change their firing patterns over intervals of sleep, and that the stereotypy of HVC projection neuron firing shows a circadian cycle during undirected singing. This daily variation in the song motor program is paralleled by small but detectable overnight changes in song acoustical structure and song variance. These variations raise the possibility that novel firing patterns generated through noisy sleep processes can be selectively reinforced during subsequent days of singing.

Disclosures: S. Moorman: None. **W.A. Liberti:** None. **L.N. Perkins:** None. **B.H. Price:** None. **T.J. Gardner:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 629.14/BBB6

Topic: F.01. Neuroethology

Support: Pew Charitable Trust

Title: Bird song syllable decoding from neural activity

Authors: *V. GILJA[1], W. JIANG[1], T. PAILLA[1], E. ARNEODO[2], T. GENTNER[3];
[1]Electrical & Computer Eng., [2]Biocircuits Inst., [3]Psychology, UCSD, La Jolla, CA

Abstract: Songbirds, just like humans, learn to produce complex vocal sequences to communicate. Their rich vocal output results from the interaction between the brain and a vocal organ that is very similar to that of humans. For these reasons, songbirds represent an ideal animal model to study the general mechanisms underlying complex, learned motor behavior. Insights gained on how birdsong is represented in higher brain areas and how those neural representations are related to motor movements are particularly desirable if we are to understand the human speech production system and to develop a human speech neuroprosthesis.
We present a discrete neural decoder that predicts different syllables of a zebra finch listening to its own song. Previous research has demonstrated that neural activity in the bird's HVC and RA is similar during listening and singing its own song. Therefore, a high-performance decoder for these auditory stimuli is likely to translate to a high-performance decoder for singing activity.

Using spiking rates from multiple neuronal units as features and a linear discriminant analysis (LDA) classifier, we identify a separability in neural space for the 7 distinct syllables of the bird song.
We compute syllable classification performance using 10-fold cross-validation and perform a grid search over window length, bin size and window onset. We achieve a peak syllable classification accuracy of 41±3% (mean +/- s.e.m; chance level is 14%). We observe a trend of increasing performance with larger window length and bin size. As we might expect, we find a tendency for higher performance with window onset close to stimulus onset. However, and more interestingly, we have found that neural spiking rates prior to stimulus onset encode information about the subsequent syllable. This seemingly acausal effect could suggest that the bird anticipates syllables of its own song before hearing them.
These initial analyses suggest that bird song syllables can be decoded from neural activity collected at the recording site. The decoding models described here do not utilize dynamical structure present in the behavior and likely to be present in the recorded neural activity. Future work will explore these dynamics and their relationship to auditory feedback and volitional motor control.

Disclosures: V. Gilja: None. **W. Jiang:** None. **T. Pailla:** None. **E. Arneodo:** None. **T. Gentner:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 629.15/BBB7

Topic: F.01. Neuroethology

Title: Identification of a neural circuit that carries viscerosensory feedback to forebrain song control nuclei

Authors: A. PERLEGOS[1], A. PERKES[1], J. MENDEZ[2], F. GOLLER[2], *M. F. SCHMIDT[1]; [1]Univ. of Pennsylvania, Philadelphia, PA; [2]Univ. of Utah, Salt Lake City, UT

Abstract: The central role of respiration during song production in birds requires delicate coordination with syringeal motor systems. Sensory feedback mechanisms must play a critical role in achieving this coordination, and input from air sac receptors in the vocal-respiratory periphery is likely to have an immediate influence on song output. This sensory information is relayed by the vagus nerve to the tractus solitarius (nTS), and could reach the forebrain song control system through projections to the inspiration related nucleus parambigualis (PAm) in the medulla which then projects to HVC via the thalamic nucleus Uvaeformis (Uva).

We report physiological evidence for the existence of this neural pathway through which viscerosensory feedback information from the respiratory brainstem can be relayed to HVC. We used short (100 ms) air pressure pulses delivered directly to the air sacs of anesthetized male zebra finches (*Taeniopygia guttata*) while recording neural activity in PAm, Uva and HVC. We recorded from PAm at 14 sites in 3 birds. Of 28 isolated single units, 18 showed short latency responses to the pressure stimulus irrespective of the phase of the respiratory cycle. We then recorded extracellular activity in HVC across 118 recording sites in 12 birds. Of the 276 units, 77 units (28%) showed a significant response to air sac stimulation with an average latency of 35 ms. To test whether viscerosensory information reaches HVC via Uva, we also recorded from antidromically identified HVC-projecting neurons in Uva. These neurons (N = 2 sites) responded vigorously to air sac stimulation without showing any significant responses to other sensory stimuli (somatosensory or visual). In one bird, we were able to record simultaneously in HVC and Uva. Thalamic responses always preceded those observed in HVC, suggesting that viscerosensory information flows from Uva to HVC. To test this directly, we inactivated Uva while recording in HVC. Preliminary data from one bird show that such inactivation completely eliminates responses in HVC. Taken together, these experiments indicate that HVC, a crucial region for song motor control, receives direct viscerosensory information from the vocal-respiratory apparatus, which could provide online feedback during singing.

Disclosures: **A. Perlegos:** None. **A. Perkes:** None. **J. Mendez:** None. **F. Goller:** None. **M.F. Schmidt:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

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Topic: F.01. Neuroethology

Support: NIH R01 - NS089679-01

NIH 1U01NS090454-01

Title: Rules for context and order in the songbird HVC.

Authors: ***W. A. LIBERTI, III**[1], E. SPENCER[1], D. P. LEMAN[1], N. PERKINS[1], D. C. LIBERTI[1], J. E. MARKOWITZ[3], T. J. GARDNER[2];
[1]Biol., [2]Boston Univ., Boston, MA; [3]Neurobio., Harvard Univ. Med. Sch., Boston, MA

Abstract: Motor actions can involve a hierarchical organization with simple or complex rules governing the sequential ordering of elementary motor units (Markowitz, 2013). However, the neural underpinnings of the serial ordering of behavior remains largely unknown. In one of the simplest cases of learned vocal-motor behavior, a male zebra finch learns to sing a single, stereotyped song. This song contains acoustic 'motifs' that are remarkably stereotyped, and on this level, the neural correlates of the behavior have been extensively studied. However, on a slightly larger time-scale of the full song performance 'bout', song is also structured: there is a well defined start, and end of song, and in the middle zebra finches can sing a variable number of motifs, and occasionally insert variations in the syllable sequence. The songbird premotor nucleus HVC is a promising place to search for 'bout-level' coding, because the nucleus is known to be involved with both the initiation and timing of song (Long, 2008; Hahnloser, 2002), and stimulation of HVC during singing can terminate, restart, or rearrange song syntax (Wang, 2008). In a closely related species, the bengalese finch, one class of neurons in HVC that project to the basal ganglia (HVC_X) displays context-dependent activity correlated to syllable repetition and transition. (Fujimoto, 2011). To date, higher order context encoding beyond the syllable level has yet to be described in zebra finches. Using cell-type specific genetically encoded calcium indicators and custom ultralight miniature head-mounted microscopes, we describe the firing patterns of excitatory projection neurons in the premotor cortical area HVC over days and weeks in the awake behaving finch. Our data reveals that calcium activity in some projections neurons in HVC correspond reliably with simple bout-level features such as song start, song termination, and motif number within a bout.
The finding that zebra finch projection neurons in HVC correlate with features of the bout-level structure will allow for the study of motor sequence organization in a particularly controlled setting, and may provide insight into the neural basis of how complex, sequential behaviors are assembled from more elementary, discrete components.

Disclosures: W.A. Liberti: None. **E. Spencer:** None. **D.P. Leman:** None. **N. Perkins:** None. **D.C. Liberti:** None. **J.E. Markowitz:** None. **T.J. Gardner:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

Location: Halls B-H

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Program#/Poster#: 629.17/BBB9

Topic: F.01. Neuroethology

Title: *In vivo* recording of song nuclei in free moving canaries

Authors: L. TROST[1], *M. L. GAHR[2], A. TER MAAT[2];
[1]Behavioural Neurobio., [2]Max Planck Inst. For Ornithology, Seewiesen, Germany

Abstract: Singing in male canaries is plastic and strongly influenced by seasonally determined levels of hormones [1, 2]. Recordings from anesthetized male canaries have revealed that photoperiodic status influences the selectivity of HVC neurons for the bird's own song [3, 4]. Testosterone implanted female canaries produce complex songs that are structurally similar to the song of reproductively active males [5]. In particular, male songs and testosterone-induced female songs are successions of repeated motor units, unlike the commonly studied zebra finches. We have succeeded in obtaining stable (multi-)unit recordings from the song nuclei RA and HVC in free moving male canaries. Up to 8 days of continuous recordings were analyzed with respect to spike activity and local field potentials. Regular firing RA units were active before calls and song syllables, including those that were part of high-frequency trills. HVC, neurons were found whose activity preceded calls and song syllables. Playback of bird's own song during surgery showed that multiunit HVC activity followed the syllable onsets, whereas it preceded the syllables during active singing. Comparison of the song related activity of HVC-neurons among male and female canaries and among canaries and zebra finches will inform about the generality of motor coding.
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Disclosures: L. Trost: None. **M.L. Gahr:** None. **A. Ter Maat:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

Location: Halls B-H

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Topic: F.01. Neuroethology

Support: Tartar Trust Fellowship OHSU to CRO

NIH Grant DC013102 to CVP

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Title: High frequency vocalizations of the Black Jacobin, a neotropical hummingbird

Authors: *C. R. OLSON[1], M. FERNÁNDEZ-VARGARS[2], C. V. PORTFORS[2], C. V. MELLO[3];
[1]Dept. of Physiol., Midwestern Univ., Glendale, AZ; [2]Washington State Univ. Vancouver, Vancouver, WA; [3]Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Hummingbirds represent a relatively recent order of vocal learning birds that have radiated into a variety of ecological niches in the Americas. Vocal learning is well represented, possibly occurring in all nine generally recognized hummingbird lineages. Here we describe the vocal behavior of Black Jacobins (*Florisuga fusca*), a representative species from the Topaz clade, recently proposed to be a sister to all other hummingbird lineages. Black Jacobins occur in the Atlantic Forest of Brazil, where they are one of the most abundant hummingbird species. Vocalizations were recorded in and around established feeders at the Museu de Biologia Mello Leitão in Santa Teresa, Espírito Santo, Brazil. We used a multichannel Avisoft recorder system with microphones capable of recording vocal signals with energy in the sonic and ultrasonic ranges. The most common vocalization was a rapid sequence of three repeats occurring over ~0.3 s., with a fundamental frequency (FF) of 12-14 KHz and strong harmonics as high as 80 KHz. A power spectrum revealed equivalent energies in the fundamental and first harmonic, and decaying energy in the upper harmonics. These triplets occurred in a number of conditions, including in flight and close to the feeders, during in flight antagonistic interactions with conspecifics, as well as when birds were perched alone away from the feeders. These vocalizations contained significant spectro-temporal complexity, consisting or rapid and periodic frequency modulations alternating with shorter unmodulated segments. There was suggestion of individual variation, which could implicate learning and the presence of cortical vocal circuitry, but further proof would require recording isolated individuals and histological study of brain circuitry. A second, call-like vocalization type occurred rarely and consisted of a downward frequency modulated utterance that occurred over ~0.02 s, at intervals of 0.5-5 s and a FF of ~10 KHz. It occurred only when birds were perched and were guarding nearby feeders. No

vocalizations were noted below 10 KHz. The frequency range for these vocalizations is remarkable in that it is above the upper limit of both the hearing and vocal frequency range thought to be present in birds in general. Tropical forests present unique acoustic challenges, including complex canopy structure and noisy environments from a diverse assemblage of species. The high frequency of Jacobin vocalizations may represent a frequency shift to better allow effective and exclusive acoustic communication in a noisy environment with a broad diversity of other hummingbird species.

Disclosures: C.R. Olson: None. **M. Fernández-Vargars:** None. **C.V. Portfors:** None. **C.V. Mello:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

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Topic: F.01. Neuroethology

Support: NIH/NINDS Grant R01 35467

Title: Androgens in the anterior forebrain pathway maintain song stereotypy in adult male canaries

Authors: *B. A. ALWARD, G. F. BALL;
Psychology, Univ. of Maryland At Col. Park, College Park, MD

Abstract: Optimal performance of social behaviors requires they be coordinated appropriately to the environmental context. During breeding contexts, male songbirds tend to produce a stable, stereotyped song that facilitates them effectively attracting a mate or repelling a competitor, while outside of breeding contexts when birds are altering their songs it becomes more variable. This is especially prevalent in open-ended vocal learners like male canaries (*Serinus canaria*), which as adults undergo enhanced song variability during the non-breeding season, but produce a highly stereotyped song during the breeding season to attract a mate. The mechanisms controlling context-dependent changes in adaptive behavioral variability are unclear. Androgens have been shown to significantly reduce vocal variability and are high during the breeding season when song is stereotyped, but low during the non-breeding season when song is highly variable. We housed male canaries on short days (SD) to simulate non-breeding conditions. Then, we used flutamide to bilaterally block androgen receptors (AR) in the lateral magnocellular nucleus of the anterior nidopallium (LMAN), a cortical-like brain region of the song control system that is known as a vocal variability generator. Immediately following

surgery birds were placed on long days (LD) to simulate the breeding season. We recorded song while birds were housed on SD and LD. Blocking AR in LMAN caused a significant increase in the acoustic variability of song and this was paralleled by a substantial increase in the acoustic variability of syllables. The results of blocking AR in LMAN are in contrast to our previous results showing that blocking AR in HVC, a sensorimotor region involved in song production, caused increased syllable usage variability and syllable sequence variability, while not affecting syllable acoustic variability. However, blocking AR in the robust nucleus of the arcopallium, a premotor brain region, leads to increased syllable acoustic variability, similarly to the current results of AR blockade in LMAN. Indeed, LMAN projects directly to RA and it is via this pathway that LMAN is thought to "inject" variability into the song control system. These results suggest AR in LMAN are important to controlling vocal variability and highlight the pleiotropic nature of steroid hormones in controlling complex social behaviors such as birdsong.

Disclosures: B.A. Alward: None. **G.F. Ball:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

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Topic: F.01. Neuroethology

Support: NINDS F32NS084909

NIMH T32 MH 89920-3

NIH RO1 MH055987

HHMI

Title: Social context modulates the expression of sequence learning in adult Bengalese finch song.

Authors: *S. SHIN, M. BRAINARD;
Physiol., UCSF, San Francisco, CA

Abstract: Male songbirds sing a more variable 'exploratory' song when they sing alone (undirected song) than when they sing to female (directed song). Undirected song is also associated with an elevated level of neural activity and neural variability within the avian anterior forebrain pathway (AFP) an avian cortico-basal ganglia analog that is crucial for normal song learning and adult song plasticity. Together, these results suggest that undirected song may

reflect a 'practice' state in which there is elevated motor exploration important for learning, while directed song reflects a 'performance' state, in which the AFP is disengaged and the current best version of song (instantiated in more primary motor structures) is produced. Consistent with this model, recently learned changes to the acoustic structure of individual syllables of adult song are partially 'reversed' when either the AFP is inactivated, or when males sing directed song (Andalman and Fee, 2009; Warren et al. 2011, Ali et al. 2013). Here, we tested whether directed song similarly modulates the expression of recently learned changes to a hierarchically distinct aspect of adult song structure: the sequencing of discrete syllables of the bird's song. We used differential reinforcement to drive changes to syllable sequencing during undirected song in adult male Bengalese finches (as in Warren at al. 2012). We then tested the effect of introducing a female, to elicit directed song, on the statistics of syllable sequencing. We found that during directed song, there was a consistent reversion of sequence learning back towards the initial baseline state. The magnitude of this reversion was correlated with the intensity of directed song, as evaluated by the concurrent effect on the variability of syllable acoustic structure. Moreover, we found that inactivation of LMAN, the outflow nucleus of the AFP, caused a similar reversion of sequence learning. These data indicate that the expression of sequence learning is subject to strong modulation by social context and suggest that this influence is mediated in part by avian basal ganglia circuitry.

Disclosures: **S. Shin:** None. **M. Brainard:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

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Topic: E.03. Basal Ganglia

Support: NIH grant R01NS094667

Pew Charitable Trust

Klingenstein Neuroscience Foundation

Simons Collaboration on the Global Brain (SCGB) Postdoctoral Fellowship

Title: Dopamine neurons encode performance error in singing birds

Authors: *V. GADAGKAR[1], P. A. PUZEREY[2], R. CHEN[2], E. BAIRD-DANIEL[2], A. FARHANG[2], J. H. GOLDBERG[2];
[1]Neurobio. and Behavior, [2]Cornell Univ., Ithaca, NY

Abstract: In reinforcement learning an animal learns through trial and error to select those actions that maximize reward. Dopamine neurons in the ventral tegmental area (VTA) mediate reinforcement by signaling reward prediction error: they are activated by better-than-predicted reward outcomes and suppressed by worse-than-predicted ones. Yet it remains unclear if dopaminergic error signals apply to behaviors such as speech or playing an instrument that are not learned for primary rewards but are instead learned by matching performance to internal goals. Songbirds use auditory feedback to learn their song, and have a dopaminergic projection from VTA to Area X, a striatal nucleus required for song learning. To test if dopamine encodes error during internally-guided performance evaluation, we recorded zebra finch VTA neurons as we induced perceived auditory error in specific song syllables using distorted auditory feedback. Here we show that a subset of VTA neurons, including those antidromically identified as projecting to Area X, encode performance error. They are suppressed after distorted syllables, consistent with a worse-than-predicted outcome, and are activated at the precise moment of the song when a predicted distortion did not occur, consistent with a better-than-predicted outcome. Error-encoding VTA neurons are a homogenous group that exhibit slow, tonic, irregular discharge similar to mammalian dopamine neurons. Yet they are intermingled with other VTA neurons that exhibit heterogeneous firing patterns, do not encode performance error, and are modulated by movement. Together, these findings identify how auditory error signals reach vocal motor circuits and, more broadly, demonstrate that principles of dopaminergic prediction error can generalize to behaviors that are not learned for primary rewards but are instead learned by comparing performance outcomes to internal goals.

Disclosures: **V. Gadagkar:** None. **P.A. Puzerey:** None. **R. Chen:** None. **E. Baird-Daniel:** None. **A. Farhang:** None. **J.H. Goldberg:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: Swiss National Science Foundation grant 31003A_127024

European Research Council under the European Community's Seventh Framework Programme (FP7/2007-2013 / ERC Grant AdG 268911)

Title: Effect of performance error size on motor correction dynamics in birdsong learning

Authors: *D. LIPKIND[1], A. T. ZAI[2], M. BATTAGLIA[2], R. H. R. HAHNLOSER[2];
[1]Dept Psychology, Hunter Col., New York, NY; [2]Inst. of Neuroinformatics, Univ. of Zurich, Zurich, Switzerland

Abstract: Adjusting motor performance to match a sensory target is crucial for the acquisition of many learned behaviors, such as speech, music, and sports. This process is commonly studied in humans and animals by inducing prediction errors via manipulation of sensory feedback. However, the mechanisms underlying learning the consequences of one's actions (and thus minimizing sensory prediction errors) are likely to be distinct from those underlying matching one's actions to a desired target (i.e., minimizing performance errors). We tested how performance errors are corrected during vocal learning in zebra finches (*Taeniopygia guttata*), by directly manipulating young birds' learning targets, without interfering with sensory feedback. We exposed birds to playbacks of an artificially designed tutor song, and once it was learned, switched the training to an altered tutor song, containing a pitch shifted version of one syllable type. We continuously recorded the birds' vocal output and tracked the developmental trajectory of individual syllables, as the birds adjusted their imitation of the first song to resemble the second song. We varied the size of the induced pitch shift across experimental groups, thus inducing larger or smaller performance errors. We found that error size had a profound effect on observed error correction trajectories. Small (one semitone) errors lead to slow and sometimes partial correction trajectories, while larger (two semitone) errors lead to abrupt and full corrections. However, further increasing error size to four semitones resulted in lack of any corrective pitch shifts towards the target. Instead, the target syllable was generated from scratch and incorporated into the song bout to replace an existing syllable. These results indicate that error size plays a crucial role in determining the assignments between individual motor actions (song syllables) and their corresponding learning targets.

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Poster

629. Bird Song: Vocal Performance and Motor Control

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Topic: E.03. Basal Ganglia

Support: NIH Grant DC02524

Title: A midbrain-basal ganglia circuit is critical to externally and internally reinforced vocal learning.

Authors: *E. HISEY, M. KEARNEY, R. MOONEY;
Neurobio., Duke Univ., Durham, NC

Abstract: Humans rely on both external and internal reinforcement to learn a variety of motor skills, from learning a video game to playing a musical instrument. Midbrain dopamine-releasing neurons that innervate the basal ganglia (BG) are known to play a key role in externally reinforced forms of learning in vertebrates. In contrast, the role of dopamine in internally reinforced forms of learning remains relatively poorly understood, due in part to the challenge of describing internally reinforced forms of learning in rodents or non-human primates. Songbirds provide a powerful model in which to explore the roles that dopamine signaling plays in internally and externally reinforced learning: as juveniles, they learn to copy a tutor song in a process that is internally reinforced; as adults, they can learn to shift the fundamental frequency (i.e., pitch) of a syllable that is targeted in a contingent manner with noise, a form of externally reinforced learning; and the pitch of the targeted syllable will return to baseline values when noise is discontinued, a process that is internally reinforced. Here we used an intersectional genetic method to ablate midbrain dopaminergic cells (VTA_X cells) that project to Area X, a region of the BG specialized for vocal learning. Though both juvenile copying and adult pitch learning were severely impaired following VTA_X cell ablation, adult recovery of baseline pitch was unaffected. In another set of experiments, we found that infusing dopamine receptor type 1 (D1R) antagonists into Area X also impaired juvenile copying and adult pitch learning, but did not affect adult recovery. These observations support the idea that D1R-mediated signaling from the VTA to Area X may be critical to learning a new motor program, but not to recovering a previously learned vocal pattern. Finally, we found that pitch-contingent optogenetic stimulation of VTA_X terminals in Area X was sufficient to reinforce lasting shifts in syllable pitch. These findings indicate that VTA_X cells and the D1Rs they activate in the BG are necessary to both internally and externally reinforced forms of learning, and that performance-contingent activation of VTA_X terminals is sufficient to reinforce learned vocal behavior.

Disclosures: **E. Hisey:** None. **M. Kearney:** None. **R. Mooney:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

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Topic: F.01. Neuroethology

Support: NIH Grant DC002524

NSF 1354962

JSPS Postdoctoral Fellowship for Research Abroad

Title: A circuit for conveying vocal timing signals to an auditory nucleus in the songbird

Authors: *M. TANAKA, R. MOONEY;
Neurobio., Duke Univ., Durham, NC

Abstract: An influential idea is that the integration of vocal motor signals with auditory feedback gives rise to error signals useful for vocal learning. The circuits that mediate sensorimotor integration important to the generation of error signals and vocal learning await elucidation. We sought to address this issue by examining the circuit that links a premotor nucleus important to song timing (i.e., HVC) to an auditory forebrain nucleus (i.e., Avalanche, or Av) in adult male zebra finches. We previously reported that this motor to auditory projection is required for song learning as well as song degradation after deafening, suggesting that it plays a role in auditory feedback-dependent vocal learning and plasticity (Hisey et al., SfN Neuroscience 2014: 565.14). To elucidate the signal that the HVC to Av projection conveys, we performed whole cell patch-clamp recordings from HVC-Av neurons in brain slices and examined their inputs using electrical and optogenetic stimulation methods. Stimulation of HVC neurons that project to a motor region (i.e., RA), which collectively encode song timing information, induced monosynaptic excitatory currents in most HVC-Av neurons. In contrast, stimulation of HVC neurons that project to a basal ganglia region (i.e., Area X) failed to elicit monosynaptic excitatory currents in any HVC-Av neuron. Moreover, stimulation of axon terminals from auditory cortical regions including Av and the nucleus interfacialis (NIf) failed to induce monosynaptic excitatory currents in HVC-Av neurons, but did evoke monosynaptic excitation in the other projection neuron types in HVC. Tracer injections showed that Av neurons project to the ventral part of the intermediate arcopallium (AIV), which contains neurons that are sensitive to perturbations of vocalization-related auditory feedback and that target midbrain dopaminergic neurons implicated in error correction (Mandelblat-Cerf et al., 2014, eLIFE 3:e02152). These results suggest that the HVC-Av projection conveys song timing information to the auditory system, which could be important to the generation of error signals in downstream auditory regions.

Disclosures: **M. Tanaka:** None. **R. Mooney:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

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NSF Graduate Research Fellowship Program

Title: The diversity of Bengalese finch song syntax and the role of auditory feedback in its complexity

Authors: S. SURENDRALAL[1], K. E. BOUCHARD[2], M. S. BRAINARD[3], *D. Z. JIN[1]; [1]Dept Physics, Pennsylvania State Univ., University Pk, PA; [2]Computat. Res. Div., Lawrence Berkeley Natl. Lab., Berkeley, CA; [3]Univ. of California at San Francisco, San Francisco, CA

Abstract: Songs of the Bengalese finch consist of variable sequences of discrete syllables. The syllable sequences follow probabilistic rules, and can be accurately described by statistical models, in particular, the partially observable Markov model (POMM) [1]. POMM is a probabilistic state transition model, with each state associated with a syllable. A syllable can be associated with multiple states. This "many-to-one mapping" from the states to the syllables distinguishes POMM from a simple Markov model, in which each syllable is associated with a single state. The number of states required for each syllable can be used to characterize the complexity of Bengalese finch songs. Using a novel method for deriving POMM from observed syllable sequences [2], we constructed the syntax of a number of Bengalese finches. We excluded syllable repetitions since these were addressed elsewhere [3]. We find diverse state transition structures, ranging from simple to complex. While some structures are composed of segments of deterministic transitions with occasional branches, others allow branching from every state. Hence there is no prototypical syntax for Bengalese finch songs. Statistical analysis shows that most songs are beyond Markov models, requiring 2 to 3 extra states for some syllables. Deafening reduces such complexity, simplifying the song syntax into Markov models in most birds. The complexity persists in others. These observations suggest that auditory feedback can induce complexity in the Bengalese finch song syntax. There are also contributions from intrinsic factors other than the auditory feedback. We suggest that the Bengalese finch song syntax is encoded in the interplay between auditory feedback and the intrinsic song generating circuitry.

[1] Jin & Kozhevnikov, PLoS Comp. Bio., 2011.
[2] Jin & Surendralal (to be published).
[3] Wittenbach, Bouchard, Brainard & Jin, PLoS Comp. Bio., 2015.

Disclosures: S. Surendralal: None. **K.E. Bouchard:** None. **M.S. Brainard:** None. **D.Z. Jin:** None.

Poster

629. Bird Song: Vocal Performance and Motor Control

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Topic: F.01. Neuroethology

Support: NIH U01NS090454

NIH R01NS089679

Title: Auditory error-signals and song destabilization in zebra finch learning and song maintenance

Authors: *N. PERKINS[1], J. MARKOWITZ[3], J. SHEN[1], D. SEMU[1], W. A. LIBERTI, III[1], D. C. LIBERTI[4], T. J. GARDNER[2];
[2]Dept. of Biol., [1]Boston Univ., Boston, MA; [3]Dept. of Neurobio., Harvard Med. Sch., Boston, MA; [4]Boston Med. Ctr., Boston, MA

Abstract: During development, zebra finches learn a distinct stereotyped song, which they are then able to consistently perform hundreds of times a day over many years. The long-term performance of this complex, learned behavior depends on auditory feedback for maintenance. Conditional aversive auditory feedback (CAAF) results in short-term adaptive plasticity of the acoustic structure of song. This externally reinforced sensory-motor learning may be related to the self-evaluation between motor performance and a sensory representations that guides normal song learning and maintenance (Leonardo & Konishi, 1999; Tumer & Brainard, 2007; Andalman & Fee, 2007). The auditory area called HVC shelf is known to be responsive to AAF (Hamaguchi et al., 2014) and provides inputs to AIV, a region that potentially drives dopamine signaling in VTA (Mandelblat-Cert et al., 2014), closing a loop from auditory circuits to a potential reinforcement signal that impacts multiple song motor nuclei as well as the song-related basal ganglia (area X).
This pathway may be critical for identifying sensory errors and modulating vocal performance adaptively. To date, there is little known about how vocal performance errors are encoded in the HVC shelf, and what role they play in song adaptation seen under CAAF. In this study, we use fiber photometry to record neural activity via genetically-encoded calcium indicators (GECIs) from identified projection neuron types in HVC and HVC shelf. AAF is probabilistically presented at a specific point in the song, triggered based on a custom low-latency syllable detector. Using a GECI targeting subpopulations of projection neurons in HVC, we are able to disambiguate CAAF responses arising in HVC shelf from potential adaptation of motor related signals in HVC. This work both expands fiber photometry methods enabling optical interfacing with specific brain regions and develops our understanding of how sensory error signals are used in maintenance and modification of learned, complex motor behaviors.

Disclosures: **N. Perkins:** None. **J. Markowitz:** None. **J. Shen:** None. **D. Semu:** None. **W.A. Liberti:** None. **D.C. Liberti:** None. **T.J. Gardner:** None.

Poster

630. Stress and Immune Function

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Program#/Poster#: 630.01/BBB19

Topic: F.04. Stress and the Brain

Support: NRF-2013R1A1A1057712

NRF-2014R1A2A2A04007391

Title: The link between anxious behaviors and habenular mast cells in social inequity-conditioned rats

Authors: ***H. LEE**, T. JUNG, W. KIM, J. NOH;
Dankook Univ., Suji-Gu, Yongin-Si, Gyeonggi-Do, Korea, Republic of

Abstract: Social equity reduces physiological reactivity to stressors, while social inequity causes emotional distress. Mast cells are immune cells that are found in the brain and affect neuronal system and emotional behavior, independent of the animal's immune status. To determine the role of neuro-immunity in emotional behaviors, we observed the brain mast cells and the anxiety-like behaviors of rats exposed to electrical foot-shock distress with different social environment. There were no differences in body weight and sucrose preference among all assigned rats. The fear memories were augmented in shock-exposed rats with nonshock- or shock- exposed conspecifics compared to solitarily shock-exposed rats. After fear conditioning, shock-exposed rats with nonshock-exposed conspecifics showed intensified anxiety-like behaviors. Importantly, we found the increase of habenular mast cell numbers in intensified anxiogenic groups. The number of mast cells in lateral habenula had a significant correlation with initial decreasing rate of anxiety-like behaviors. Taken together, present study demonstrates that social inequity induces anxiety-related emotional alteration and mast cell changes in the habenula. It provides evidences for the affective behavioral importance of mast cell links in social stress and suggests the functional role of habenular mast cells for helping to relieve a social stress caused exaggerated biopsychological responses. (Supported by NRF-2013R1A1A1057712 and NRF-2014R1A2A2A04007391)

Disclosures: **H. Lee:** None. **T. Jung:** None. **W. Kim:** None. **J. Noh:** None.

Poster

630. Stress and Immune Function

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Topic: F.04. Stress and the Brain

Support: NIH Grant R01-MH093473

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Title: Microglia actively recruit inflammatory monocytes to the brain to promote anxiety-like behavior with Repeated Social Defeat.

Authors: *M. D. WEBER, C. SAWICKI, D. B. MCKIM, J. P. GODBOUT, J. F. SHERIDAN; Ohio State Univ., Columbus, OH

Abstract: Neuroinflammation affects mood, cognition, and behavior and is linked with the etiology of psychiatric disorders, including anxiety and depression. Repeated social defeat (RSD) is a murine stress model that recapitulates many of the immune and behavioral outcomes of stress. For instance, RSD activates fear/stress neurocircuitry, accompanied by microglial activation, increased pro-inflammatory signaling and the promotion of anxiety. We have reported that a key part of RSD-induced anxiety is the trafficking of inflammatory Ly6C^{hi} monocytes from the bone marrow to fear and threat appraisal center of the brain. We hypothesize that the site-specific trafficking of Ly6C^{hi} monocytes to the brain is caused by active recruitment directed by resident microglia. To address this, we used the colony-stimulating factor (CSF)-1 antagonist, PLX5622, to eliminate microglia and determined their role in RSD induced macrophage recruitment to the brain and the induction of anxiety. In these experiments C57/BL6 mice were feed control or PLX5622 diet for 14 days and remained on diet with exposure to RSD. We first confirmed that mice feed the PLX5622 diet had a 99% reduction in brain microglia by cell flow cytometry, histology, and mRNA analyses. PLX5622 intervention alone, however, had no effect on myeloid cells in the bone marrow or in circulation. In addition, PLX5622 intervention and the elimination of resident microglia had no effect on stress-dependent increase in myelopoiesis in bone marrow and enhanced accumulation of Ly6C^{hi} monocytes in circulation. PLX5622 intervention did not affect the stress induced up regulation of vasculature adhesion molecules (ICAM and VCAM) but blocked vascular expression of IL-1 receptor. Importantly, microglia elimination abrogated the recruitment of Ly6C^{hi} monocytes to the brain with stress. This blockade was associated with attenuated neuroinflammatory signaling with stress (reduced IL-1β, CCL2, CCR2, IL-6, TNFα). Moreover, microglia elimination and the absence of inflammatory monocytes prevented the induction of anxiety-like behavior with stress. Taken

together, these data indicate that repeated social defeat activates microglia, which selectively recruit inflammatory monocytes to the brain that augment neuroinflammatory signaling and promote anxiety-like behavior.

Disclosures: M.D. Weber: None. **C. Sawicki:** None. **D.B. McKim:** None. **J.P. Godbout:** None. **J.F. Sheridan:** None.

Poster

630. Stress and Immune Function

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Topic: A.07. Developmental Disorders

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University Grants Commission (20-29(12)/2012(BSR),

ICMR SRF (45/13/2014-Nan/BMS),

Title: Resveratrol suppresses neuroinflammation in the experimental paradigm of ASD

Authors: *R. BHANDARI[1,2], A. KUHAD, 160014[3];
[1]Panjab Univ., Chandigarh, India; [2]Pharmacol., [3]Univ. Inst. of Pharmaceut. Sci., Chandigarh, India

Abstract: Objective: Neuronal dysfunction caused by neuroinflammation triggered by the stimulation of matrix metalloproteinases and the subsequent release of pro-inflammatory cytokines, as a result of oxidative stress and mitochondrial dysfunction, is one of the probable mechanisms involved in the pathogenesis of autism spectrum disorders (ASD). The aim of the present study was to explore the ameliorative potential of resveratrol on neuroinflammation in the experimental paradigm of neuroinflammatory model of ASD in rats. **Method:** 1M Propanoic acid (PPA)(4µl) was infused over 10 minutes into the anterior portion of the lateral ventricle to induce ASD like symptoms in rats. Resveratrol (5, 10 and 15 mg/kg) was administered starting from the 2nd day of the surgery and continued upto 28th day. Rats were tested for various behavioural paradigms such as social interaction, stereotypy, locomotor activity, anxiety, novelty, depression, spatial learning, memory, repetitive and pervasive behaviour between the 7th day and 28th day. In addition, biochemical tests for oxidative stress, mitochondrial complexes, TNF-alpha and MMP-9 were also assessed. **Results:** Treatment with resveratrol for

four weeks restored, significantly and dose dependently, all the neurological, sensory, behavioural, biochemical and molecular deficits in PPA induced autistic phenotype in rats. **Conclusion**: The major finding of the study is that resveratrol restored the core and associated symptoms of autistic phenotype by suppressing oxidative-nitrosative stress, mitochondrial dysfunction, TNF-alpha and MMP-9 expression in PPA induced ASD in rats. Therefore, resveratrol might serve as an adjunct potential therapeutic agent for amelioration of neurobehavioural and biochemical deficits associated with autism spectrum disorders. ***Keywords:*** Autism spectrum disorders (ASD), resveratrol, neurobehavioural, oxido-nitrosative stress, TNF-alpha, MMP-9

Disclosures: **R. Bhandari:** None. **A. Kuhad:** None.

Poster

630. Stress and Immune Function

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Program#/Poster#: 630.04/BBB22

Topic: F.04. Stress and the Brain

Support: 15SDG22430017

P20GM103641

Title: Physical versus psychological stress-induced inflammation: a putative role of estrogen in stress susceptibility

Authors: *J. E. FINNELL, C. M. LOMBARD, C. M. MOFFITT, S. K. WOOD; Pharmacology, Physiology, and Neurosci., Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: Physical exposure to or witnessing social stressors is known to result in the development of depression, which has been associated with elevated peripheral and central inflammation. While women are more susceptible to developing depression than men, the mechanism by which this occurs is unknown. A major system implicated in depression is dysregulation in the locus coreuleus (LC)-norepinephrine (NE) system. Inflammation and estrogen have both been shown to increase NE release from the LC, suggesting a mechanism of increased stress sensitivity in females. Therefore, we utilized a model of social defeat stress to determine differences in stress-induced inflammation between (1) physical (defeated intruder) vs. psychologically stressed (witness) male rats, (2) female intact vs. ovarectomized (OVX) witness rats, and (3) peripheral vs. central responses to social defeat or witness stress in males and females. Male Sprague Dawley rats were randomly assigned into control, intruder or witness

groups. Female Sprague Dawley rats were assigned into control/witness and intact /OVX groups. Intruders were placed into the home cage of a novel Long Evans retired breeder (resident) for 15 mins on 5 consecutive days. Each intruder was paired with a witness, which was placed behind a plexi-glass partition in the resident cage for the duration of the intruder defeat exposure. 4 days after the 5th stress/control exposure, a sucrose preference test was administered and 24 hrs later all stressed rats were re-exposed to the defeat environment in the absence of the resident (15 mins) followed immediately by plasma/tissue collection. Bio-plex analysis of circulating cytokines indicated moderate increases in cytokine expression in male intruders, while male witnesses did not differ from controls. The cytokine response of both intact and OVX female witnesses was significantly elevated from controls, although baseline inflammation was greater in OVX controls. Analysis of sucrose preference indicated that only intact female witnesses exhibited anhedonia. These data indicate that females, regardless of intact or OVX exhibit a more sensitized peripheral inflammatory response to witnessing social stress as compared to males. However, anhedonia was only observed in intact females suggesting that this depressive-like behavior is not driven by peripheral inflammation. Alternatively, ongoing studies are assessing whether intra-LC inflammation contributes to the depressive-like responses in intact females. Furthermore, this enhanced behavioral sensitivity may be reliant upon the effects of estrogen in the stress sensitive LC. Support: 15SDG22430017 and P20GM103641

Disclosures: **J.E. Finnell:** None. **C.M. Lombard:** None. **C.M. Moffitt:** None. **S.K. Wood:** None.

Poster

630. Stress and Immune Function

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Topic: F.04. Stress and the Brain

Support: CIBERSAM

COMPLUTENSE UNIVERSITY OF MADRID

INSTITUTO DE INVESTIGACIONES SANITARIAS HOSPITAL 12 DE OCTUBRE

Title: Antiinflammatory role of perivascular macrophages in a model of depression induced by stress in rats

Authors: *A. SAYD[1,3,2], K. MACDOWELL[2,3], D. MARTIN-HERNANDEZ[2,3], J. CASO[2,3], A. VARGAS[2], J. LEZA[2,3], J. MADRIGAL[2,3], L. ORIO[2], B. GARCIA-BUENO[2,3];
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Abstract: Perivascular macrophages (PVM) are hematopoyetic cells that migrate to the brain perivascular space modulating the interactions between the immune and central nervous systems (CNS). Previously, their depletion with the icv administration of the pro-apoptotic drug clodronate encapsulated in liposomes increased the vascular production of the proinflammatory prostaglandin PGE_2, the release of ACTH, corticosterone and fever, induced by the intravenous administration of Lipopolysaccharide. Further studies also demonstrated a decrease in the synthesis of the antiinflammatory prostaglandin 15d-PGJ_2. With this background, we decide to explore the mechanisms involved in the anti-inflammatory profile of PVM by depleting them in a model of depression induced by chronic mild stress (CMS) exposure in rats. Our results showed an increase of cytokines TNFα, IL-1 and IL-6 at mRNA levels in the prefrontal cortex of the groups where the PVM were depleted, as well as in the protein levels of nuclear factor NF-κB, the enzymes proinflammatory iNOS, COX-2 and m-PGES-1 and their product PGE_2. A concomitant decrease of the 15d-PGJ_2 mediator was also observed. In addition we also checked whether the depletion of PVMs could regulate the expression of molecules implicated in the leukocyte traffic and infiltration in the CNS in our CMS model. Thus the mRNA levels of the chemokines MCP-1, fractalkine and the adhesion molecule VCAM appeared increased in the animals without PVMs. In summary our results could suggest a potential anti-inflammatory role for PVMs in a depression model chronic stress-induced as CMS.

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Poster

630. Stress and Immune Function

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Program#/Poster#: 630.06/BBB24

Topic: F.04. Stress and the Brain

Title: The influence of stress and inflammation on amyloid-beta production.

Authors: *M. J. EIMERBRINK[1], J. WHITE[1], J. PETERMAN[1], R. PENDRY[1], C. HAGEN[1], H. MOORE[1], M. CHUMLEY[2], G. BOEHM[1];
[1]Psychology, [2]Biol., Texas Christian Univ., Fort Worth, TX

Abstract: The connection between inflammation and various disease pathologies is strongly confirmed in the existing literature, as is the connection between stress and immune function. Here we present data from a series of studies designed to examine the relationship between stress, inflammation, and amyloid-beta production. First we examined how two different stressors, repeated social defeat and social isolation, influence the pro-inflammatory cytokine response to lipopolysaccharide (LPS). Results from this study provide evidence that both paradigms are capable of increasing the inflammatory response to LPS. Additionally, we found that stress can increase expression of the pro-inflammatory primer, high mobility group box 1 (HMGB1). Following this, we studied the effect of stress on inflammation-induced amyloid-beta production in a non-transgenic model, using C57BL6/J mice. After completion of a stress protocol, animals were treated with either LPS or saline once per day for seven consecutive days. Results show that the treatment with LPS increases expression of amyloid-beta in the hippocampus, and that stress exacerbates LPS-induced expression of hippocampal amyloid-beta. We also show that LPS-induced elevations in hippocampal amyloid-beta are sufficient to induce cognitive deficits in a contextual fear conditioning paradigm. Taken together, our results support the hypothesis that stress and inflammation can interact to increase amyloid-beta production.

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Poster

630. Stress and Immune Function

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Topic: F.04. Stress and the Brain

Support: NARSAD

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Herman Dana Foundation

Title: Altered blood mononuclear cell transcriptional reactivity accompanying the development of postpartum depression

Authors: *T. GOLTSER[1], T. SHIMONOVITZ[2], S. KLAR[1], L. CANETTI[1], E. GALILI[3], I. SHAHAR[1], D. PEVZNER[1], O. OZ[1], I. VASHDI[1], N. FRIEDMAN[4], D. HOCHNER-CELNIKIER[2], R. SEGMAN[1];
[1]Hadassah Hebrew U, Jerusalem, Israel; [2]Hadassah Mt. Scopus - Hebrew Univ. Med. center,

Jerusalem, Israel; [3]Hadassah Univ. Hospital, The Herman-Danna Div. of Pediatric Psychiatry, Dept. of Psychiatry, Hadassah - Hebrew Univ. Med. Ctr., Jerusalem, Israel; [4]Sch. of Computer Sci. and Engineering, Hebrew Univ., Jerusalem, Israel

Abstract: Background: Genome scale comparison of mononuclear cell transcriptional engagement following a common trigger of delivery, allows an unbiased window into differential systemic immune alterations during the triggering of postpartum depression. Methods: Reproductive steroid hormones and blood mononuclear transcriptional signature immediately following delivery were compared between mothers developing a depressive episode and those who remained unaffected upon prospective follow up. Results: Bioinformatic analyses demonstrated altered immune - inflammatory activation among mothers developing a depressive episode. Differently expressed transcripts enriched Pathways supporting altered mononuclear cell activation accompanying the development of a depressive episode. Reproductive hormones did not show significant differences among depressed mothers. Discussion: Genome scale expression changes immediately following delivery implicate altered mononuclear cell immune activation among mothers developing a depressive episode upon long term follow. Immune activation may be mechanistically involved in depression pathogenesis through neural - immune signaling. This work was supported by a NARSAD independent investigator award, an Israel Science Foundation Grant 1563-08 and the Herman Dana Foundation

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Poster

630. Stress and Immune Function

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Topic: F.04. Stress and the Brain

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Title: Electrophysiological alterations of synaptic inputs converging on area CA3 the hippocampus in a model of prenatal infection.

Authors: *C. SOTO[1], E. GÁLVAN[2];
[1]Farmacobiología, [2]CINVESTAV, Sede Sur, Mexico, Mexico

Abstract: Maternal infections during pregnancy have been identified as a risk factor for the development of neurological and psychiatric disorders in the progeny; a condition that is mimicked by prenatal exposure of rats to lipopolysaccharide (LPS). In this model, the resultant offspring will experience impaired learning and psychotic-like behaviors. Thus, we explored the changes in the synaptic strength of glutamatergic inputs converging on area CA3 of the hippocampus as well as the interneuron-mediated inhibition of animals exposed to prenatal infection (LPS administration on gestational days 15 and 16, 100 μg/Kg). A histological analysis revealed a structural disorganization of the dentate granule cell layer and pyramidal cell layer of the hippocampus of animals prenatally exposed to LPS. The strength of the glutamatergic inputs, assessed by mean of input-output curves (I-O curves), indicate an increase in the excitability ratio of the perforant path to dentate granule cells (PP-DG) synapse and the PP-CA3 pyramidal cells (PP-CA3) synapse. The increased excitability was also found in the mossy fiber-CA3 synapse (MF-CA3). Paired-pulse protocols were used to unmask changes in the interneuron-mediated inhibition and short-term synaptic enhancement of these synapses. Compared to control, the PP-DG, PP-CA3, and MF-CA3 synapses exhibited a decreased index of depression. Conversely, the three synaptic inputs exhibited an increased index of facilitation. These electrophysiological alterations indicate an imbalance in the inhibition/excitation ratio as a consequence of the exposure to LPS. As the synaptic interactions between DG and area CA3 take part in the transfer of the somatosensorial information arriving from the entorhinal cortex, the synaptic imbalance observed in this study, may represent the mechanistic basis underlying the cognitive impairment triggered by prenatal infections.

Disclosures: C. Soto: None. **E. Gálvan:** None.

Poster

630. Stress and Immune Function

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Topic: F.04. Stress and the Brain

Support: NIH GRANT 5R25GM061151

Title: Effects of sleep deprivation on gut microbiota in *Drosophila* menlanogaster

Authors: *Y. ORTIZ-CASTELLNO[1], N. RODRIGUEZ-GOMEZ[2], J. F. RUIZ[2], M. G. DOMINGUEZ-BELLO[3], C. LEUCIANO-MONTALVO[2], J. L. AGOSTO-RIVERA[2];

[1]Biol., Univ. of Puerto Rico At Rio Piedras, Caguas, PR; [2]Biol., Univ. of Puerto Rico At Rio Piedras, San Juan, PR; [3]Med., New York Univ. Langone Med. Ctr., New York, NY

Abstract: Sleep loss are common in today's society. Sleep deprivation can have serious consequences on health affecting cardiovascular, immune and the central nervous system (CNS), among others. Interestingly, sleep difficulties have also been associated with intestinal microbiota dysbiosis and changes in protein expression of the CNS. Early classical experiments transferring cerebrospinal fluid (CSF) from sleep-deprived animals into rested non-deprived animals established that the homeostatic control of sleep involved the accumulation of sleep-inducing substances. Later studies identified fragments of bacterial cell wall products called muramyl peptides (MPs) as important sleep-inducing substances. Although it was known that MPs could be produced by phagocytosis of gram-negative and gram-positive bacteria, whether the bacterial source came from opportunistic infections, endogenous bacteria or contamination has been the subject of a long-standing debate and speculation. Here we take advantage of the sequencing tools available in our genomic era and the Drosophila melanogaster, to shine a light into this issue. We show for the first time that: 1) sleep deprivation leads to a global decrease in the number of gut microbes and alters the relative abundance of specific bacterial strains and; 2) these changes depend on the translational repressor *Pumilio* since knock-down of this gene prevent these alterations; 3) rearing the fruit flies with broad-spectrum antibiotics produced a decrease in total sleep. Taken together, these results indicate that gut microbes are affected by sleep deprivation and may play a role in sleep homeostasis. Given the alarming increases in the prevalence of chronic sleep deprivation and related disorders in our society and the accessibility of gut microbes, our findings could provide the basis for both diagnostic tools for sleep conditions as well as novel treatments.

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Poster

630. Stress and Immune Function

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Topic: F.04. Stress and the Brain

Support: 1R21MH096224-01

Title: Acute stress induces a protracted priming of amygdalar microglia: a role for the alarmin HMGB1, CD200R and NLRP3

Authors: *M. G. FRANK, R. M. BARRIENTOS, L. K. FONKEN, L. R. WATKINS, S. F. MAIER;
Univ. of Colorado, Boulder, CO

Abstract: Stress sensitizes the neuroinflammatory as well as the microglial pro-inflammatory response to subsequent immune challenges. Much of this prior work has focused, in large part, on hippocampal microglia. Recent evidence suggests that the amygdala is particularly sensitive to the pro-inflammatory effects of stress, however, it is unknown whether stress primes the pro-inflammatory response of amygdalar microglia to subsequent immune challenges. To address this question, we examined the effects of acute stress on amygdalar microglia activation markers in vivo as well as the pro-inflammatory response of isolated amygdalar microglia ex vivo. Male Sprague-Dawley rats were exposed to inescapable tailshock (IS; 100 shocks, 1.6 mA, 5 sec ITI) or served as home cage controls (HCC). 8 days post-IS exposure, amygdala was dissected and microglia immunophenotype was characterized using real time RT-PCR. In addition, protein levels of the alarmin HMGB1 were measured. In the amygdala, we found that IS increased HMGB1 protein and mRNA, NLRP3 mRNA and NFKBIA mRNA, but decreased mRNA levels of CD200R and MHCII. In addition, IS increased the expression of the microglia inhibitory ligands CD200 and CX3CL1. Notably, the ratio of CX3CR1 to CX3CL1 (1.09) in IS subjects did not significantly differ from HCC (1.22). However, exposure to IS induced a profound downward shift in the ratio of CD200R to CD200 (3.91) compared to HCC (13.28). Importantly, IS exposure failed to affect pro-inflammatory cytokine (IL-1 beta, TNFa and IL-6) expression suggesting that stress primes rather than activates amygdalar microglia. To test this notion, microglia were isolated from amygdala 8 days after exposure to IS using a Percoll density gradient. Amygdalar microglia were then treated with LPS (0, 1, 10 and 100 ng/ml) for 2h ex vivo and pro-inflammatory cytokine expression measured. Prior exposure to IS potentiated the pro-inflammatory cytokine (IL-1 beta, TNFa) response of amygdalar microglia indicating that IS primes amygdalar microglia to subsequent immune challenges. The present results suggest that acute stress induces a protracted priming of amygdalar microglia. Further, these priming effects may be the result of stress-induced dis-inhibition of microglia through down-regulation of the microglial inhibitory receptor CD200R.

Disclosures: M.G. Frank: None. **R.M. Barrientos:** None. **L.K. Fonken:** None. **L.R. Watkins:** None. **S.F. Maier:** None.

Poster

630. Stress and Immune Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 630.11/CCC3

Topic: F.04. Stress and the Brain

Support: Israel Ministry of Health Chief Scientist

Herman Dana Foundation.

Title: Mononuclear cell transcriptional reactivity following first methylphenidate challenge among drug naïve children with Attention Deficit and Hyperactivity Disorder

Authors: *R. SEGMAN[1], T. GOLTSER DUBNER[1], A. MELTZER[2], G. BODENHEIMER[2], A. SHARON[2], R. GIESSER[2], L. KALMAN[2], A. SHALEV[2], L. CANETTI[1], E. GALILI[2]; [1]Hadassah Univ. Hosp., Jerusalem, Israel; [2]The Herman-Danna Div. of Pediatric Psychiatry, Dept. of Psychiatry, Hadassah - Hebrew Univ. Med. Ctr., Jerusalem, Israel

Abstract: Background: Exposure to stimulant drugs has been increasing in recent years among children and adolescents, during critical periods of brain development. Understanding their mechanism of action, or long term molecular effects, is hindered by lack of access to relevant brain cells in humans. Methods: Children diagnosed with Attention Deficit Hyperactivity Disorder (ADHD) underwent a computerized assessment of their clinical response to an initial methylphenidate trial. Quantitative changes in continuous computerized performance measures was compared between the baseline drug naïve state and peak methylphenidate effect, and correlated with simultaneous mononuclear cell genome scale transcriptional reactivity. Results: Transcriptional changes following methylphenidate exposure among children demonstrating a concomitant prospectively documented clinical response, point to predictive clinical drug response biomarkers, as well as implicate potentially relevant mechanistic drug targets. Conclusions: Prospectively documented systemic transcriptional reactivity following initial methylphenidate exposure among previously drug naïve children diagnosed with ADHD, may offer a surrogate venue offering insight into molecular pathways of methylphenidate action. This work was supported by an Israel Ministry of Health Chief Scientist grant and the Herman Dana Foundation.

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Poster

630. Stress and Immune Function

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Program#/Poster#: 630.12/CCC4

Topic: F.04. Stress and the Brain

Support: CIBERSAM

Complutense University of Madrid

Instiuto de Investigaciones Sanitarias Hospital 12 de Octubre

Title: The antioxidant nuclear factor Nrf2 pathway dysregulation and its modulation by antidepressants in chronic mild stress rats

Authors: *D. MARTÍN HERNÁNDEZ[1,2], Á. GONZÁLEZ BRIS[1,2], A. SAYD GABÁN[1,2], K. MACDOWELL MATA[1,2], B. GARCÍA BUENO[1,2], J. MUÑOZ MADRIGAL[1,2], J. LEZA CERRO[1,2], J. CASO FERNÁNDEZ[1,2];
[1]Pharmacol., Complutense Univ. of Madrid, Madrid, Spain; [2]CIBERSAM, Madrid, Spain

Abstract: *Introduction:* patients with major depression who are otherwise medically healthy have activated inflammatory pathways. It has been described that depression is not only escorted by inflammation but also by induction of multiple oxidative/nitrosative stress pathways. Nevertheless, there are finely regulated mechanisms involved in preserving cells from damage, such as the nuclear factor Nrf2.
Aims: to explore in a depression-like model the Nrf2 pathway in the prefrontal cortex (PFC) and the hippocampus of rats and to analyze which classic antidepressants affect the antioxidant activity of the Nrf2 pathway.
Methods: male Wistar rats were exposed to chronic mild stress (CMS) and some of them were treated with desipramine, escitalopram or duloxetine. We studied the expression in the PFC and hippocampus of upstream and downstream elements of the Nrf2 pathway and the oxidative damage induced by the CMS.
Results: after exposure to a CMS protocol, in the PFC, there is an inhibition of upstream and downstream elements of the Nrf2 pathway. Moreover, antidepressant treatments, particularly desipramine and duloxetine, are able to recover some of these elements and to reduce the oxidative damage induced by the depression model. In the hippocampus however, Nrf2 pathways are not that affected and antidepressants do not have many actions.
Conclusions: Nrf2 pathway is differentially regulated by antidepressants in the PFC and hippocampus. The Nrf2 pathway is involved in the oxidative/nitrosative damage detected in the PFC after CMS exposure. However, it seems that Nrf2 is not very involved in the effects caused by the CMS in the hippocampus.

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Poster

630. Stress and Immune Function

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Program#/Poster#: 630.13/CCC5

Topic: F.04. Stress and the Brain

Support: CIHR Grant

Title: Probiotic modulation of brain cytokine changes elicited by social defeat and a bacterial endotoxin in male mice

Authors: J. K. SZYSZKOWICZ[1], M. SEDRAK[1], E. KERR[1], H. ANISMAN[1], *M.-C. AUDET[2,1];
[1]Neurosci., Carleton Univ., Ottawa, ON, Canada; [2]Inst. of Mental Hlth. Res., Ottawa, ON, Canada

Abstract: Social stressors and bacterial endotoxins known to elicit anxiety- and depressive-like behaviours in mice increase pro-inflammatory cytokines in stress-sensitive brain regions and disturb microbial communities in the gastrointestinal tract. Appreciable evidence indicates that interventions that target the gut microbiome may modulate peripheral and brain cytokines and come to affect neurochemical processes and behaviour. The aim of the current study was to examine whether promoting a healthy gut microbiota via a probiotic treatment would prevent variations of the pro-inflammatory cytokines interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α in the prefrontal cortex (PFC) elicited by a social stressor or an immune challenge. CD-1 male mice received a probiotic mixture (*L. helveticus* and *B. longum*; 1 x 10^9 colony forming units/day) or a placebo daily for 3 weeks. A subset of mice then experienced an acute (15 min) or a chronic (15 min on each of 10 consecutive days) social defeat stressor or their respective control conditions whereas another subset received an intraperitoneal injection of the bacterial endotoxin lipopolysaccharide (LPS; 10 μg) or a saline solution (*n*=10/group). Locomotor activity was monitored for 90 minutes following the stressor or immune challenges after which blood and brain tissue from the PFC were collected for the determination of plasma corticosterone levels and gene expression of pro-inflammatory cytokines, respectively. Elevations of plasma corticosterone levels and of prefrontal IL-6 mRNA expression elicited by the social stressors and LPS were not prevented by the 3-week probiotic treatment. Curiously, IL-1β expression in the PFC was altered only when the social stressors or LPS were administered to probiotic-treated mice. These results suggest that gut microbial influences on brain inflammatory activation elicited by stressor or endotoxin challenges might be cytokine-specific.

Disclosures: J.K. Szyszkowicz: None. **M. Sedrak:** None. **E. Kerr:** None. **H. Anisman:** None. **M. Audet:** None.

Poster

630. Stress and Immune Function

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Program#/Poster#: 630.14/CCC6

Topic: F.04. Stress and the Brain

Support: NIHMS704781

Title: Sympathetic nervous system contributes to enhanced corticosterone levels following chronic stress.

Authors: *A. KULP[1], S. LOWRANCE[2], E. FUSCO[2], H. KENNEDY[2], M. RUSS[2], N. ASHCHERKIN[2], A. PARKER[2], J. JOHNSON[2];
[1]Biomed. Sci., [2]Neurosci., Kent State Univ., Kent, OH

Abstract: Exposure to chronic stress often elevates basal circulating glucocorticoids during the circadian nadir and leads to exaggerated glucocorticoid production following exposure to subsequent stressors. While glucocorticoid production is primarily mediated by the hypothalamic-pituitary-adrenal (HPA) axis, there is evidence that the sympathetic nervous system (SNS) can affect diurnal glucocorticoid production by direct actions at the adrenal gland. Experiments here were designed to examine the role of the HPA and SNS in enhancing corticosterone production following chronic stress. Rats were exposed to a four-day stress paradigm or control conditions then exposed to acute restraint stress on the fifth day to examine corticosterone and ACTH responses. Repeated stressor exposure resulted in a small increase in corticosterone, but not ACTH, during the circadian nadir, and also resulted in exaggerated corticosterone production 5, 10, and 20 min following restraint stress. While circulating ACTH levels increased after 5 min of restraint, levels were not greater in chronic stress animals compared to controls until following 20 min. Administration of astressin (a CRH antagonist) prior to restraint stress significantly reduced ACTH responses but did not prevent the sensitized corticosterone response in chronic stress animals. In contrast, administration of chlorisondamine (a ganglionic blocker) returned basal corticosterone levels in chronic stress animals to normal levels and reduced early corticosterone production following restraint (up to 10 min) but did not block the exaggerated corticosterone response in chronic stress animals at 20min. These data indicate that increased SNS tone contributes to elevated basal and rapid glucocorticoid production following chronic stress, but HPA responses likely mediate peak corticosterone responses to stressors of longer duration. Future studies are aimed to investigate the role brain cytokines play in the over-activation of the SNS following chronic stress and to use Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) techniques to activate major control regions for sympathetic output such as the rostral ventrolateral medulla and measure glucocorticoid production.

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Poster

630. Stress and Immune Function

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 630.15/CCC7

Topic: F.04. Stress and the Brain

Title: Corticosterone pre-treatment sensitises microglial inflammatory responses: non-classical neuroimmune actions

Authors: *J. LIU[1], S. MUSTAFA[2,3], M. R. HUTCHINSON[1,3];
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Abstract: Corticosterone (CORT), the predominant end product of the hypothalamus pituitary adrenal axis in mice, is a stress response hormone which is classically anti-inflammatory. Recent evidence has shown that CORT can mediate the neuroimmune sensitisation effects of stress in mice, which could contribute to the neuroimmune involvement in stress-related disorders. This study investigates the conditions in which CORT can prime the immune system, by measuring the effects of CORT pre-treatment on BV2 mouse microglia-like cells and RAW264.7 mouse macrophage-like cells on subsequent immune responses to Lipopolysaccharide (LPS) exposure in vitro.Results from this current study demonstrated both anti- and pro-inflammatory actions of CORT. CORT was anti-inflammatory in all measures at a high physiological dose (500nM). Low-dose (50nM) CORT however increased IL-1β conversion and release in BV2 but not RAW264.7 cells, only after removal of CORT pre-treatment before addition of LPS. This sensitisation was not found in IL-6 responses to the same dose of LPS. Low-dose CORT also increased nuclear translocation of NF-κB p65, a pro-inflammatory transcriptional factor, while retaining the ramified morphology of BV2 cells during LPS treatment. These results suggest that mild elevations in CORT, a common occurrence in stress-related disorders such as depression, causes selective adaptations in microglia to over-respond to a second immune challenge in a non-classical manner, thus partially explaining both pro- and anti-inflammatory effects of CORT reported in the literature. CORT-induced mRNA expression changes in the Toll-like receptor 4, inflammasome, and steroid receptor pathways in response to LPS administration will be explored, to further explain the mechanisms behind this selective sensitisation in BV2 cells.

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Poster

630. Stress and Immune Function

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Topic: F.04. Stress and the Brain

Support: 2010 NARSAD Young Investigator Award

German Research Foundation Grant RE 2911/5-1

University of Colorado Boulder

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Title: Microbiome and behavior: Immunization with a heat-killed preparation of the environmental bacterium Mycobacterium vaccae promotes stress resilience in mice

Authors: ***C. A. LOWRY**[1], P. H. SIEBLER[1], N. C. DONNER[1], J. T. MORTON[1], D. G. SMITH[1], J. M. KOPELMAN[1], K. R. LOWE[1], K. J. WHEELER[1], J. H. FOX[1], J. E. HASSELL, Jr.[1], B. N. GREENWOOD[1], C. JANSCH[1,4], A. LECHNER[4], D. SCHMIDT[4,5], N. USCHOLD-SCHMIDT[5], A. M. FUCHSL[5], D. LANGGARTNER[5,6], F. R. WALKER[6,7], M. W. HALE[7], G. LOPEZ PEREZ[1,2], W. VAN TRUEREN[2,8], A. GONZALEZ[8,3], A. L. HALWEG-EDWARDS[3], M. FLESHNER[1,9], C. L. RAISON[9,10,11], G. A. ROOK[10], S. D. PEDDADA[11], R. KNIGHT[8,12], S. O. REBER[12];
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Computat. Biol. Br., Natl. Inst. of Envrn. Hlth. Sci., Research Triangle Park, NC; [12]Clin. for Psychosomatic Med. and Psychotherapy, Univ. of Ulm, Ulm, Germany

Abstract: The prevalence of inflammatory diseases is increasing in modern urban societies. Inflammation increases risk of stress-related pathology; consequently, immunoregulatory or anti-inflammatory approaches may protect against negative stress-related outcomes. To test this hypothesis, we immunized (3 x weekly immunizations) adult male mice with a heat-killed preparation of the immunoregulatory bacterium, *Mycobacterium vaccae* (NCTC 11659), or vehicle and then evaluated gut microbiome and defensive behavioral responses during exposure to the chronic subordinate colony housing (CSC) model of chronic psychosocial stress, as well as immunologic responses and anxiety-like behavioral responses following the CSC procedure. Briefly, CSC exposure consisted of co-housing four subordinate mice with one dominant resident mouse for a period of 19 days. Subordinate mice were moved to the cage of a new, dominant male aggressor on days 8 and 15. Single housed mice were used as controls. We show that stress disrupts the homeostatic relationship between the microbiota and the host, resulting in exaggerated inflammation, as assessed by histological damage to the colon, stress-induced exaggeration of dextran sulfate sodium-induced colitis (a murine model of inflammatory bowel disease) and increased release of interferon-γ and interleukin-6 from freshly isolated mesenteric lymph node cells stimulated with anti-CD3 antibody in vitro. Repeated immunization with a heat-killed preparation of *M. vaccae* reduced subordinate, flight, and avoiding behavioral responses to a dominant aggressor in a murine model of chronic psychosocial stress when tested 1-2 wk following the final immunization. Furthermore, immunization with *M. vaccae* prevented stress-induced spontaneous colitis and, in stressed mice, induced anxiolytic or fear-reducing effects as measured on the elevated plus-maze, despite stress-induced gut microbiota changes characteristic of gut infection and colitis. Immunization with *M. vaccae* also prevented stress-induced aggravation of colitis in a model of inflammatory bowel disease. Depletion of regulatory T cells negated protective effects of immunization with *M. vaccae* on stress-induced colitis and anxiety-like or fear behaviors. Consistent with long-term effects of *M. vaccae* immunization on neural systems implicated in control of emotional behavior, immunization with *M. vaccae* increased tryptophan hydroxylase 2 mRNA expression in the dorsal raphe nucleus, regardless of prior stress exposure. These data provide a framework for developing microbiome- and immunoregulation- based strategies for prevention of stress-related pathologies.

Disclosures: **C.A. Lowry:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Mycobacterium vaccae used in this study was provided under a Materials Transfer Agreement between Immodulon Therapeutics, UK and the University of Colorado Boulder. **P.H. Siebler:** None. **N.C. Donner:** None. **J.T. Morton:** None. **D.G. Smith:** None. **J.M. Kopelman:** None. **K.R. Lowe:** None. **K.J. Wheeler:** None. **J.H. Fox:** None. **J.E. Hassell:** None. **B.N. Greenwood:** None. **C. Jansch:** None. **A. Lechner:** None. **D. Schmidt:** None. **N. Uschold-Schmidt:** None. **A.M. Fuchsl:** None. **D. Langgartner:** None. **F.R. Walker:** None. **M.W. Hale:** None. **G. Lopez Perez:** None. **W. Van Trueren:** None. **A. Gonzalez:** None. **A.L. Halweg-Edwards:** None. **M. Fleshner:** None. **C.L. Raison:** None. **G.A. Rook:** None. **S.D. Peddada:** None. **R. Knight:** None. **S.O. Reber:** None.

Poster

630. Stress and Immune Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 630.17/CCC9

Topic: F.04. Stress and the Brain

Title: The affect of repeated stress exposure on microglial phagocytosis

Authors: *D. F. BARNARD, J. D. JOHNSON;
Kent State Univ., Kent, OH

Abstract: Microglia are the innate immune cell within the central nervous system. They are highly motile, phagocytic cells that actively scan their microenvironment and are capable of releasing various signaling molecules including inflammatory cytokines (e.g. IL-1, TNF-alpha, and IL-6) that are potent inducers of both behavioral and physiological processes. Recent studies have indicated that stress has profound impacts on microglia morphology, density, proliferation, and activation state, which implicate their role in certain pathologies. Our lab has previously shown that exposure to chronic stress induces anhedonic behavioral responses in Fischer rats approximately 5-days after stressor onset. To examine the activation state of microglia at this time, rats were exposed to a four-day chronic mild stress protocol. Twenty-four hours after the last stressor rats were euthanized and microglia isolated from the hippocampus were placed in culture with or without further stimulation. After 4h in culture basal IL-1, TNF-alpha, and IL-6 protein production were not altered in microglia collected from stressed animals compared to microglia collected from controls. Interestingly, after LPS stimulation IL-1 production was attenuated in microglia collected from stressed animals while there were no changes in the production of TNF-alpha or IL-6. Current studies are examining the effect of stress on phagocytosis, an important function of microglia in clearing debris from the brain such as amyloid-beta, by incubated microglia with latex beads coated with fluorescently-labeled rabbit IgG. Prior research has suggested that chronic stress induces microglia to transition from a surveillance state to a more active state characterized by increase in microglial activation marker Iba-1. Here, we report no increase in basal IL-1 production following 5-days of stressor exposure and a suppression in IL-1 production following LPS challenge.

Disclosures: D.F. Barnard: None. **J.D. Johnson:** None.

Poster

630. Stress and Immune Function

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Topic: F.04. Stress and the Brain

Support: NIH Grant MH084970

NIH Grant MH109484

Title: Repeated social defeat stress shifts peripheral immune function in rats: potential impact on basolateral amygdala neurons

Authors: *S. MUNSHI, J. A. ROSENKRANZ;
Rosalind Franklin Univ. of Med. & Sci., North Chicago, IL

Abstract: Psychological stress can induce depression and anxiety, and numerous studies suggest involvement of the immune system in these effects. The amygdala is sensitive to stress and may be an intermediary in the link between immune function and depression and anxiety. Repeated social defeat stress (RSDS) is a powerful model of social stress with known impact on immune function. The peripheral immune system includes pro-inflammatory and anti-inflammatory aspects that, as a whole, may exert very different influences on behavior. The aim of this study is to (1) determine the effect of RSDS on the balance between pro-inflammatory and anti-inflammatory aspects of peripheral immune function, and (2) determine if a shift in this balance causes a change in amygdala function. Adult male Sprague Dawley rats underwent RSDS by exposure to the resident-intruder model using unfamiliar aggressor Long Evans rats for five consecutive days, or control handling. After three days the following were evaluated: (a) peripheral circulating T-cell counts and their intracellular cytokine profile (Th1, Th2) by flow-cytometry, (b) pro- and anti-inflammatory serum cytokine levels by ELISA, and (c) anxiety-like behavior in the open field test (OFT). RSDS decreased the frequency of CD4+ T-cells with no changes in that of the CD8+ T-cells. Frequency of CD4+ T-cells positive for the Th2-like cytokine was reduced without any alteration in those of the CD8+ T-cells. Additionally, RSDS caused changes in many different serum cytokines. OFT showed that RSDS induced an increase in anxiety-like behavior. To test whether a pro-inflammatory state can induce a change in the amygdala physiology, *in vivo* extracellular electrophysiological recordings of the basolateral amygdala (BLA) were performed in a different set of rats that underwent peripheral immune challenge with interleukin-1β. Interleukin-1β caused a trend of a time-dependent alteration of the evoked local field potential as well as alteration of the spontaneous neuronal firing-rate in the BLA. Taken together, these data suggest that RSDS shifts immune balance, and that a shift in immune balance impacts BLA neuronal activity. This could contribute to the effects of RSDS on

anxiety behavior. The results have implications in understanding how chronic stress might alter BLA physiology by recruitment of the peripheral immune system.

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Poster

630. Stress and Immune Function

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Topic: F.04. Stress and the Brain

Support: 1R43MH105048-01

Title: Selective inhibition of soluble tumor necrosis factor in a rodent model of depression with increased inflammation: a potential approach to treating treatment resistant depression

Authors: *L. N. EIDSON[1], C. J. BARNUM[1], B. DUKE[1], Y. YANG[1], J. CHANG[1], S. D. KELLY[1], M. E. S. RODRIGUES[1], A. H. MILLER[1], R. J. TESI[2], M. G. TANSEY[1]; [1]Emory Univ., Atlanta, GA; [2]FPRT bio, Scranton, PA

Abstract: Approximately 7 million of the 20+ million sufferers of Major Depressive Disorder (MDD) do not respond to classical anti-depressant treatment termed treatment resistant depression (TRD). Sickness behavior and depression share multiple characteristics, suggesting that inflammation may contribute to a depressive phenotype. Indeed, cytokines, most notably tumor necrosis factor (TNF), are elevated in depressed patients, and a recent trial demonstrated that a subset of TRD patients with elevated levels of inflammatory c-reactive protein (CRP) improve following treatment with infliximab, a non-selective TNF antagonist. Unfortunately, commercially available non-selective TNF antagonists have an unacceptable safety profile; including increased risk of immunosuppression and demyelinating neurologic disease due to action at both soluble TNF (solTNF) and transmembrane TNF (tmTNF). While solTNF drives chronic inflammatory disease, tmTNF facilitates innate immunity to infection and myelination. Here we tested whether administration of a novel, brain permeable, selective inhibitor of solTNF, XPro1595, attenuates depressive- and anxiety-like behaviors and inflammation in a mouse model of depression. Adolescent male and female C57BL/6 mice were bred in house and subjected to chronic psychological stress for 15 consecutive days (P35 - P50) then tested for a depressive-like phenotype (sociability and sucrose preference). Mice were then subject to an additional 15 consecutive days of stress while receiving XPro1595 (10 mg/kg; sc) or vehicle (saline) treatment every third day, and then retested for a depressive- and anxiety-like phenotype (sociability, sucrose preference, and open field). Plasma inflammation was measured every 2 weeks during

stress testing, and hippocampus and prefrontal cortex were collected at end-point to measure peripheral and central inflammation. Our data indicate that chronic adolescent stress induces depressive-like behavior that can be ameliorated by solTNF antagonism using XPro1595. Sexual dimorphisms exist in several measures, and solTNF inhibition in the absence of stress induces abnormal social behavior. Together, these data will lend insight into how to select a target population for sequestration of solTNF by XPro1595 to improve quality of life for individuals suffering from TRD. [Funding by 1R43MH105048-01].

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Poster

630. Stress and Immune Function

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Topic: F.04. Stress and the Brain

Support: NIH Grant R21 MH092667

Penn State Institute of the Neurosciences

Penn State Social Science Research Institute

Title: Asthma during adolescence contributes to adult anxiety behavioral and neurobiological phenotype

Authors: *J. I. CAULFIELD[1,2,3], M. J. CARUSO[1,3], R. A. BOURNE[1], S. A. CAVIGELLI[1,2,3]; [1]Biobehavioral Hlth., [2]Grad. Program in Neuroscience, Huck Inst. for Life Sci., [3]Ctr. for Brain, Behavior, and Cognition, The Pennsylvania State Univ., University Park, PA

Abstract: Adolescence is a developmental period sensitive to perturbations that can affect adult neuronal and behavioral processes associated with internalizing disorders, like anxiety and depression. Asthma is a common chronic health challenge during adolescence, affecting 9% of U.S. adolescents, and often comorbid with anxiety and depression. However, little is known about the neurobehavioral impacts of this chronic adolescent challenge. Microglia, the resident immune cells of the brain, become activated in response to peripheral insult, and their over-activation has been implicated in neuropsychiatric disorder development. The mechanism

underlying the comorbidity of asthma and internalizing disorders, and the involvement of microglia in this relationship, has not been established. The present study implemented a mouse model of chronic adolescent asthma to investigate the physiological properties that underlie the connection between asthma and anxiety, as well as the potential involvement of microglia. Three experimental groups, consisting of male and female BALB/c mice, were designed to examine the components of an asthma attack: (1) "Airway inflammation" via repeated house dust mite extract (HDM) exposure; (2) "Labored breathing" via methacholine (MCH) exposure; and (3) "Airway inflammation and Labored breathing" via both HDM and MCH exposure. As adults, MCH animals demonstrated an anxious phenotype, spending 30% less time on open arms of the elevated plus maze compared to non-MCH animals. These mice also had decreased serotonin transporter gene expression in the brainstem, which is consistent with findings supporting low serotonin transporter activity as a risk for developing anxiety. Additionally, MCH animals demonstrated elevated serotonin receptor 1a, mineralocorticoid, and Cd11b expression in the hippocampus compared to non-MCH mice (Cd11b expression indicates microglia activation). HDM-exposed mice exhibited 50% less basal circulating corticosterone levels compared to controls. Preliminary results for hippocampal gene expression of Cd11b revealed a sex difference in HDM-MCH animals, with females exhibiting higher levels. The results of the present experiment indicate that clinical symptoms of chronic asthma, particularly labored breathing, during adolescence lead to increased adult anxiety-related behavior and brain function.

Disclosures: J.I. Caulfield: None. **M.J. Caruso:** None. **R.A. Bourne:** None. **S.A. Cavigelli:** None.

Poster

630. Stress and Immune Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 630.21/CCC13

Topic: F.04. Stress and the Brain

Support: NIH grant R21MH092667

PSU Institute for Neurosceince

PSU Social Science Research Institute

Title: Hippocampal gene networks associated with anxiety- and depression-related behavior caused by adolescent asthma symptoms in male BALB/cJ mice

Authors: *M. J. CARUSO[1], H. M. KAMENS[2], W. J. HORTON[3], R. A. BOURNE[4], A. AUGUST[7], L. C. KLEIN[1], R. H. BONNEAU[5], T. CRAIG[6], S. A. CAVIGELLI[1]; [1]Biobehavioral Hlth., Pennsylvania State Univ., State College, PA; [2]Biobehavioral Hlth., Pennsylvania State Univ., State College PA, PA; [3]Animal Sci., [4]Biol., Pennsylvania State Univ., State College, PA; [5]Microbiology and Immunology; Pediatrics, [6]Med. & Pediatrics; Allergy, Asthma & Immunol., Pennsylvania State Univ., Hershey, PA; [7]Microbiology and Immunol., Cornell Univ., Ithaca, NY

Abstract: Asthma is a stressful chronic disease with high prevalence among adolescents. Moreover, increased rates of internalizing disorders are reported in humans with asthma. The current study aimed to identify alterations in adult anxiety- and depression-like behaviors, glucocorticoid production, and hippocampal gene transcription resulting from symptoms of adolescent allergic asthma. To mimic adolescent allergic asthma symptoms, we exposed male BALB/cJ mice to inhaled house dust mite extract to stimulate lung inflammation, and/or to inhaled methacholine to stimulate bronchoconstriction. Basal glucocorticoid and proinflammatory cytokine production and anxiety- and depression-like behavior were measured in adulthood. Hippocampal gene expression was then measured using RNA sequencing. Transcriptional data were analyzed for asthma-induced changes by: (1) differential gene expression, and (2) gene co-expression using Weighted Gene Co-expression Network Analyses (WGCNA). Adolescent bronchoconstriction caused increased adult anxiety- and depression-like behavior (less time on open arms of elevated plus maze and increased immobility in forced swim test) compared to animals that did not experience bronchoconstriction. Bronchoconstriction was also associated with altered neuroendocrine function as indicated by a reduction in basal glucocorticoid levels. Alternatively, mice that experienced airway inflammation had elevated proinflammatory cytokine levels, but there was no difference in basal glucocorticoid production, anxiety- or depression-like behavior. Bronchoconstriction-induced behavioral changes were accompanied by transcriptional enrichment of mechanistic target of rapamycin (mTOR) signaling pathways in the hippocampus. Prior research indicates that mTOR signaling is involved in regulation of protein translation and synaptogenesis. WGCNA revealed a module of co-expressed genes whose expression profile was associated with both anxiety-like behavior and experimentally-induced bronchoconstriction. *Rictor*, an essential subunit of mTOR protein complex 2 (mTORC2), was identified as an important regulator of gene expression in this module. Neuronal mTORC2 regulates dendritic morphology and synaptic function. Results suggest that anxiety-related behavior that results from adolescent bronchoconstriction is associated with aberrant hippocampal mTOR signaling. These findings raise the possibility that aberrant hippocampal synaptic plasticity, associated with mTOR signaling, contributes to increased anxiety-related behavior observed after repeated bronchoconstriction during development.

Disclosures: **M.J. Caruso:** None. **H.M. Kamens:** None. **W.J. Horton:** None. **R.A. Bourne:** None. **A. August:** None. **L.C. Klein:** None. **R.H. Bonneau:** None. **T. Craig:** None. **S.A. Cavigelli:** None.

Poster

630. Stress and Immune Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 630.22/CCC14

Topic: F.04. Stress and the Brain

Support: CONACyT 243419

FIS/IMSS/PROT/G/14/1299

Title: Early life stress activates glial cells in the hippocampus but attenuates cytokine secretion in response to an immune challenge in rat pups

Authors: L. SAAVEDRA - PIMENTEL[1], B. FENTON - NAVARRO[3], *L. TORNER[2];
[1]Ctr. de investigacion Biomedica de Michoacan, [2]Inst. Mexicano Del Seguro Social, Morelia, Mexico; [3]Facultad de ciencias medicas y biologicas, Univ. Michoacana de San Nicolas de Hidalgo, Morelia, Mexico

Abstract: Early life stress (ELS) affects permanently the development of the central nervous system (CNS) and increases the vulnerability to develop psychological disorders such as anxiety and depression in adulthood. These diseases are accompanied by inflammatory processes in the brain. Plus, exposure to infections during early stages sensitizes the neuroimmune system and increases the risk to develop psychopathology. Recently we showed that maternal separation (MS) activates microglial cells and induces the expression of IL-1β in the hippocampus. However it is not known how ELS affects the response of the neuroimmune system to an immune challenge. Therefore we studied the effect of MS followed by the administration of lipopolysaccharide (LPS) on the morphology of microglial cells and astrocytes in CA3 region and the Hilus of the hippocampus and on cytokine release at postnatal day (PN) 15. Male Sprague Dawley rat pups were subjected to MS (3h/day, PN1 to PN14) or remained undisturbed at the mother´s nest (Control). LPS (1mg/Kg bw) or vehicle was administered on PD14 to half of each group and they were subjected to the open field (OF) test 1h later. Some groups were sacrificed on PD15 for histological analyses. Other groups of pups were sacrificed 90 min after LPS injection (PN14) and the blood samples and hippocampi were collected. LPS treatment reduced the locomotion of control and MS pups in the OF test 1h later and reduced their body weight gain 24h later. LPS - induced Corticosterone levels were similar in both Control and MS groups. MS induced a mild decrease, but LPS alone or combined with MS reduced more the total number of microglial cells. Activation of microglial cells was observed in hippocampal CA3 and hilus of MS - Veh, it was increased in control - LPS, and was highest in MS - LPS pups. Astrocyte density was reduced in SM - Veh, Control - LPS and reduction was maximal in SM - LPS pups in CA3 area and hilus. In contrast, LPS induced an increased secretion of plasmatic IL-1β, TNFα, IL-6, and of hippocampal IL-1b. However, MS imposed an attenuation of the

cytokine response to LPS in the periphery and reduced IL-1 β protein levels in the hippocampus. We conclude that although the neuroimmune cells are highly activated by MS and LPS, stress induces the attenuation of the hippocampal and peripheral cytokine response by an unidentified mechanism. (CONACyT 243419).

Disclosures: L. Saavedra - Pimentel: None. **B. Fenton - Navarro:** None. **L. Torner:** None.

Poster

631. Risk Factors for Brain Disorders

Location: Halls B-H

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Program#/Poster#: 631.01/CCC15

Topic: F.04. Stress and the Brain

Support: MH108286

MH073030

MH099910

MH104184

Title: Paternal stress programming by epigenetic crosstalk: Brain-specific changes in the histone code via sperm microRNA

Authors: *J. CHAN, N. LEU, N. V. BHANU, B. A. GARCIA, T. L. BALE;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Parental exposures to environmental insults such as stress, diet, drugs or toxins have been linked with increased risk of neuropsychiatric disease in subsequent generations. Whereas the mechanisms by which maternal insults can impact fetal neurodevelopment have been widely investigated, how the paternal environment impacts future offspring health is not well understood. Rodent models, where males do not participate in offspring rearing, provide an exciting model for examining the unique epigenetic germ cell contribution to offspring development. Recent studies have shown that paternal exposure to a variety of perturbations can impact offspring behavior and physiology, and have identified changes in histone modifications, DNA methylation, and/or small non-coding RNAs in sperm as potential mechanisms of transmission. We have developed a paternal stress model in which offspring exhibit significantly blunted stress reactivity – a feature of many neuropsychiatric disorders. Mechanistically, we identified 9 microRNA (miRs) increased in paternal sperm following stress exposure. Zygote microinjection of these miRs recapitulated the blunted stress phenotype, providing evidence for a

functional role for sperm miRs. Interestingly, RNA-seq analyses of the adult hypothalamus from zygote-microinjected offspring revealed dramatic transcriptional repression, suggesting an upstream epigenetic repressive mechanism that is developmentally programmed by paternal stress. However, how paternal experiences program lasting changes in the offspring brain and what these changes are is not known. Using single-cell amplification, we found that sperm-derived miRs can regulate maternal mRNA stores in the zygote, and may direct developmental programming by targeting expression of epigenetic regulators. Indeed, RNA-seq analyses of embryonic day 12.5 brains demonstrate a robust, brain-specific reprogramming in the zygote-microinjected embryos that include shifts in expression of many chromatin modifiers. Histone mass spectrometry of these embryonic brains confirm altered abundances of several histone post translational modifications, suggesting sperm miRs can alter the histone code that regulates offspring neurodevelopment. Ongoing studies examine the maintenance and mechanistic contribution of developmentally altered histone marks to the adult hypothalamic phenotype. These studies confer the importance of paternal experiences in influencing offspring health, and offer an exciting and novel epigenetic mechanism by which the male germ cell can reprogram offspring brain development. Supported by MH073030, MH099910, MH104184, MH108286.

Disclosures: **J. Chan:** None. **N. Leu:** None. **N.V. Bhanu:** None. **B.A. Garcia:** None. **T.L. Bale:** None.

Poster

631. Risk Factors for Brain Disorders

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Program#/Poster#: 631.02/CCC16

Topic: F.04. Stress and the Brain

Support: MH091258

MH087597

MH099910

MH104184

Title: Regulation of male risk and female resilience to neurodevelopmental disorders by placental H3K27me3

Authors: ***T. L. BALE**[1], C. O'DONNELL[2], B. NUGENT[2];
[1]Dept Animal Biol, [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Many neurodevelopmental disorders are more prevalent in boys than girls. In our mouse model of early prenatal stress (EPS), stress exposure during the first week of gestation imparts long-term neurodevelopmental programming deficits in male offspring resulting in hypersensitivity to stress, cognitive impairments, and alterations in metabolic programming. The placenta, a fetally-derived organ reflecting fetal sex chromosome complement, acts as an arbitrator between the mother and fetus, providing necessary factors for early fetal neurodevelopment. Thus, sex differences in placental function may dramatically influence sex bias in vulnerability to prenatal insults. We previously identified the X-linked, stress sensitive, nutrient sensor O-linked-N-acetylglucosamine (OGT) as a placental biomarker of prenatal stress. OGT escapes X-inactivation in the placenta, providing females with two copies and males with one copy. Placental-specific reduction of OGT recapitulates the neurodevelopmental and metabolic impairments associated with EPS exposure. As OGT is known to modify several epigenetic regulators associated with histone methylation, we hypothesized that sex differences in OGT mediate sex differences in histone methylation and promote sex-specific programs of gene expression. Using ChIP-Seq, biochemistry, and RNA-Seq in mouse placentas with trophoblast-specific OGT reduction, we found that OGT determines genome-wide sex differences in H3K27me3 and gene expression in placental trophoblasts. Further, RNA-Seq of the embryonic hypothalamus revealed that reducing OGT copy number in the female placenta masculinized the expression of key genes associated with hypothalamic development and function in the female brain, suggesting that placental OGT contributes to sex differences in brain development. Females have far more H3K27me3 in placental trophoblasts than males, thus we hypothesize that female-biased epigenetic repression protects females from prenatal insults such as EPS. To test this hypothesis, we reduced H3K27me3 in female placentas to male-like levels using trophoblast-specific genetic manipulations of the H3K27me2/3 methyltransferase, EZH2. We predict that females with decreased placental EZH2 will be vulnerable to the effects of EPS, and display sensitized HPA axis function, metabolic deficiencies, and cognitive impairments in adulthood. These studies, aimed at elucidating the basic biological differences between male and female developmental programs, will bring us closer to fully understanding the etiology of sex-biased neurodevelopmental disorders.

Disclosures: **T.L. Bale:** None. **C. O'Donnell:** None. **B. Nugent:** None.

Poster

631. Risk Factors for Brain Disorders

Location: Halls B-H

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Program#/Poster#: 631.03/CCC17

Topic: F.04. Stress and the Brain

Support: MH091258

MH087597

MH099910

MH104184

Title: Prenatal stress alters exosome microRNA content and delivery to the developing brain

Authors: *B. M. NUGENT, T. L. BALE;
Dept. of Biomed. Sci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Understanding how prenatal stress alters the fetal milieu is important for identifying mechanisms involved in perturbations of brain programming, which are often associated with neurodevelopmental disorders. In our well-established mouse model, male offspring exposed to early prenatal stress (EPS) have altered hypothalamic pituitary axis (HPA) programming, resulting in increased stress sensitivity and dysregulation in hypothalamic metabolism, similar to endophenotypes identified in boys with autism and men with early-onset schizophrenia. Importantly, our EPS protocol occurs during the first week of gestation, prior to brain development and thus likely alters programming of peripheral tissues in addition to altering neurodevelopmental processes. Previously, our lab found that gene sets important for endo- and exosomal cellular processes are down regulated in the male placenta in response to EPS, suggesting that EPS alters fetal exosome signaling. Exosomes are small vesicles secreted locally and into the bloodstream by most tissues. Exosomes transfer proteins, microRNAs, and other signaling factors between cells and tissues as a means of short and long-distance communication. Importantly, exosomes cross the blood-brain barrier in adults, but their potential to enter and participate in the programming of the developing brain has not been explored. To determine if EPS alters exosome signaling from peripheral tissues to the brain, we are using near infrared whole-animal imaging and confocal microscopy of fluorescently-labeled exosomes in brain tissue sections to track the destination(s) and cellular localization of peripheral exosomes. In addition, microRNAs from control and EPS-exposed fetal serum exosomes were quantified by small RNA-Seq. Concurrently, we performed RNA-Seq on mRNA from hypothalamic punches from the same fetuses used for exosomal microRNA analysis and used bioinformatic approaches to determine if predicted microRNA targets were altered in the brain. Robust changes in exosomal microRNA cargo following EPS that corresponded to changes in hypothalamic gene expression were detected, particularly in male fetuses. To determine if EPS-altered peripheral exosomes contribute to neurodevelopmental programming, we collected exosomes from EPS exposed neonates and cross-injected them into control animals. We predict that injection of EPS-related exosomes recapitulates aspects of EPS exposure in control neonatal mice.

Disclosures: **B.M. Nugent:** None. **T.L. Bale:** None.

Poster

631. Risk Factors for Brain Disorders

Location: Halls B-H

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Program#/Poster#: 631.04/CCC18

Topic: F.04. Stress and the Brain

Support: MH073030

MH099910

MH104184

MH108286

MH091258

MH087597

Title: Peripubertal stress reprograms the paraventricular nucleus transcriptome and disrupts HPA axis responsiveness only during pregnancy.

Authors: K. E. MORRISON[1], *C. N. EPPERSON[2], T. L. BALE[1];
[1]Dept. of Biomed. Sci., Univ. of Pennsylvania, Philadelphia, PA; [2]Psychiatry, Univ. of Pennsylvania Perelman Sch. of Med., Philadelphia, PA

Abstract: Adverse childhood experiences, including those during the puberty transition, are one of the greatest predictors for affective disorder presentation for women. As the puberty transition is marked by hormonal changes and ensuing reorganization of the brain and periphery, it may represent a window of vulnerability for adversity to result in long-term reprogramming. Periods of hormonal flux in the female lifespan, including pregnancy and postpartum, exacerbate the risk for affective disturbances and promote hypothalamic-pituitary-adrenal (HPA) axis dysregulation, a key feature of affective disorders. However, little is understood as to how stress experienced during the pubertal transition alters ongoing brain maturation and its interaction with the hormonal changes that occur during pregnancy. We hypothesized that stress during peripuberty, a time of dynamic hormonal change, would enact reprogramming that would interact with a later period of hormonal change, pregnancy, to elicit dysregulated HPA axis responsiveness. Female mice were exposed to chronic stress from postnatal days 21-34, and we examined endpoints in adults related to stress pathway regulation. Corticosterone release following acute restraint stress was assessed prior to pregnancy, during pregnancy, and postpartum. Peripubertal stress resulted in a blunted corticosterone response only during pregnancy. This suggests that HPA programming by peripubertal stress intersects with the state of pregnancy to uncover dysregulation. To investigate the mechanisms by which peripubertal stress interacts with

pregnancy to produce this outcome, we examined peripheral and central regulators of the HPA axis. There was no effect of peripubertal stress on peripheral regulators, including relevant gene expression in the pituitary, adrenal, and placenta, during pregnancy. Transcriptomic analysis of the paraventricular nucleus of the hypothalamus (PVN) during pregnancy via RNA-sequencing revealed a long-term reprogramming of gene expression by peripubertal stress, including widespread changes in immediate early gene expression and their target genes, suggesting the involvement of an upstream epigenetic mechanism. Effects of peripubertal stress on histone marks in the PVN during peripuberty and pregnancy were examined. To understand how pregnancy unmasks HPA axis dysregulation, we conducted pharmacological manipulation of allopregnanolone availability within the PVN. Together, these studies confirm that the pubertal transition is a time of susceptibility for stress to enact central reprogramming and HPA axis dysregulation, particularly during later periods of hormonal flux.

Disclosures: K.E. Morrison: None. **C.N. Epperson:** None. **T.L. Bale:** None.

Poster

631. Risk Factors for Brain Disorders

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 631.05/CCC19

Topic: A.07. Developmental Disorders

Support: EU-AIMS

BRAINVIEW

Title: Characterization of resting state network topography in newborn infants at high and low risk of developing neurodevelopment disorders

Authors: *J. CIARRUSTA, J. O'MUIRCHEARTAIGH, R. DIMITROVA, I. POTE, L. CORDERO-GRANDE, A. PRICE, E. HUGHES, J. KANGAS, E. PERRY, D. BATALLE, J. HAJNAL, T. ARICHI, D. MURPHY, G. MCALONAN;
King's Col. London, London, United Kingdom

Abstract: Family history of psychiatric conditions can be a significant risk factor for developing Neurodevelopmental Disorders (NDD). MRI studies of brain networks from older patients with NDD reveal subtle alterations in ‘resting-state’ networks, however it is unknown whether such abnormalities are already present at birth. We piloted the use of a multiband (MB) excitation sequence to improve temporal resolution and characterization of high frequency activity of fMRI data in neonates. 10 low risk (LR)(43 weeks) and 6 high risk (HR)(44 weeks) were recruited as

part of the EU-AIMS Brain Imaging in Babies project. HR categorization was based on family history of psychiatric conditions (autism, ADHD and maternal MDD). fMRI data were acquired with a Philips 3T Achieva scanner and a multi-slice echo planar Imaging (15min, resolution 2mm) with multiband (MB) excitation (MB factor 9; TR 0.37s). Data pre-processing was performed using FSL. Single subject data sets were denoised following independent component analysis (ICA) to identify motion, MB and physiological artefact components. Data was warped into an age-appropriate template space, and concatenated group ICA was performed with a dimensionality of 25. Group means and differences were then identified using a general linear model (controlling for age) and the dual regression method. Group ICA identified a rich repertoire of resting state networks across subjects, which was consistent with those described in the literature. There was no significant difference between the LR and HR subjects in any of the identified resting state networks (see figure 1). At this time, we did not identify a significant difference in their spatial representation when comparing infants at HR and LR of developing NDD. However this was a small exploratory sample and was not powered to reveal subtle differences between groups in network architecture. Recruitment of a larger cohort is on-going and we hope the rich temporal and spatial information available using this method may promote understanding about the early pathophysiology of neurodevelopmental disorders.

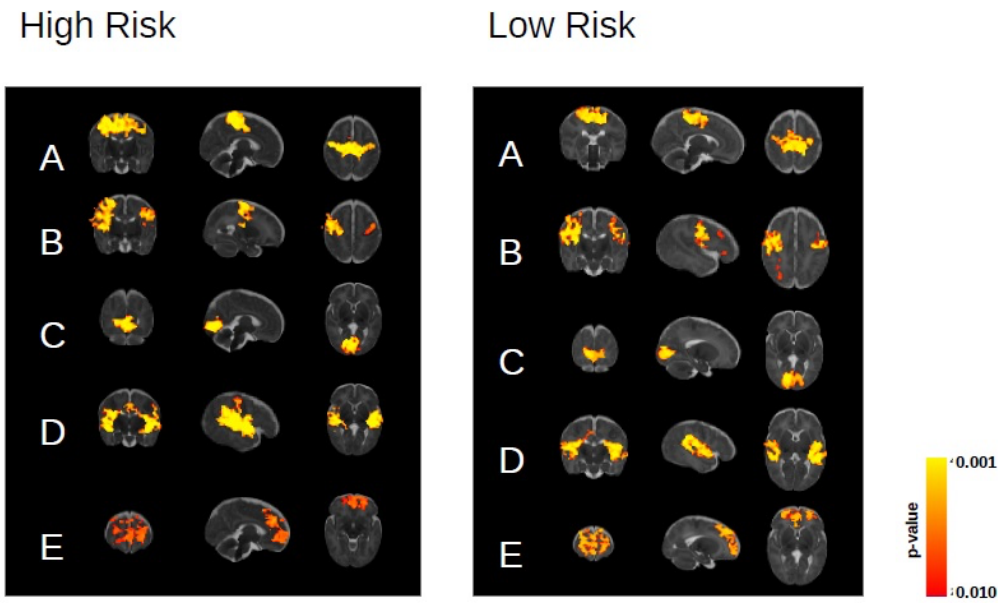

Figure 1. Neonatal Resting State Network
Coronal, sagittal and transverse views of resting state networks overlaid on a neonatal brain template (41 weeks) for low risk and high risk subjects at a threshold of $p<0.01$ (corrected). Representative networks were observed in the somatosensory region (A), motor cortex (B), visual cortex (C), auditory cortex (D) and prefrontal cortex (E).

Disclosures: **J. Ciarrusta:** None. **J. O'Muircheartaigh:** None. **R. Dimitrova:** None. **I. Pote:** None. **L. Cordero-Grande:** None. **A. Price:** None. **E. Hughes:** None. **J. Kangas:** None. **E.**

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Poster

631. Risk Factors for Brain Disorders

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Program#/Poster#: 631.06/CCC20

Topic: A.07. Developmental Disorders

Title: Chronic stress during adolescence experienced by parents can increase risk for autism spectrum disorder in offspring: a proposed animal model.

Authors: *G. BOERO[1], M. PISU[3], F. BIGGIO[2], M. SERRA[2];
[1]Dept. of Life and Enviromental Science, Section of Neurosci., Univ. of Cagliari, Monserrato, Italy; [2]Univ. of Cagliari, Cagliari, Italy; [3]CNR, Inst. of Neurosci., Cagliari, Italy

Abstract: Adverse life experiences during critical periods for CNS development may cause epigenetic changes that alter hormones pattern and receptors expression that impact brain circuits. These modifications have been associated with increased risk for autism in offspring; for instance, women exposed to childhood abuse experience were more likely to have sons with autism spectrum disorder (ASD) than women unexposed (Roberts et al., 2013). The aim of this research was to characterize a naturalistic animal model of ASD. We tested if the exposure parents to a chronic mild stress during adolescence (social isolation from weaning) could reproduce some ASD symptoms in the subsequent generation. Offspring of socially isolated rats shown a decrease in plasma oxytocin levels, a peptide that plays a crucial role in social behavior that has been shown to be altered in ASD patients. In agreement, in the resident-intruder test these rats spent more time in cage exploration and self-grooming activity rather than in the interaction with the intruder animal. Moreover, offspring of socially isolated parents showed behavioral inflexibility in the Morris water maze test during the probe trial as demonstrated by an increase in the time spent in target zone (i.e. the latency to escape from this quadrant searching platform elsewhere inside the arena). Conversely, learning and spatial memory was not affected; in agreement hippocampal BDNF levels were increased in offspring of socially isolated rats. Furthermore, these animals showed an increase of plasma corticosterone and ACTH levels, suggesting an increase of HPA axis basal activity; nevertheless, this increase was not related to an anxiety-like behavior in the elevated plus maze test. Social deficit, behavioral perseveration, HPA hyperactivity, low oxytocin and high BDNF levels are peculiar characteristics of some established genetic models of autism and ASD patients. Thus, offspring of socially isolated animals meets the attribute of face validity for ASD animal model. The advantage of this model

could be to allow the study of molecular mechanisms underlying ASD, without genetic modifications that could trigger cell adaptation mechanisms and lead to changes in other brain circuits, not involved in pathology. References: Roberts AL et al. Association of maternal exposure to childhood abuse with elevated risk for autism in offspring JAMA Psychiatry 2013

Disclosures: G. Boero: None. **M. Pisu:** None. **F. Biggio:** None. **M. Serra:** None.

Poster

631. Risk Factors for Brain Disorders

Location: Halls B-H

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Program#/Poster#: 631.07/CCC21

Topic: F.04. Stress and the Brain

Support: FAPESP #2015/18773-1

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FAPESP-BEPE #2012/21401-0

CNPQ

CAPES

FAEPA

FMRP-USP

Title: Experimental and clinical findings on the hypothalamus-pituitary-adrenal axis. Stress as a risk factor for neuro-psychopathologies

Authors: *E. H. UMEOKA[1], C. W. BAES[1], L. D. GODOY[2], N. C. B. BARROCA[2], M. S. S. UMEOKA[2], J. ANTUNES-RODRIGUES[2], N. GARCIA-CAIRASCO[2], M. F. JURUENA[1,3,4]; [1]Neurosci. and Behavioral Sci., Ribeirão Preto Sch. of Med. - Univ. of São Paulo, Ribeirao Preto, Brazil; [2]Physiol., Ribeirão Preto Sch. of Med. - Univ. of São Paulo, Ribeirão Preto, Brazil; [3]Inst. of Psychiatry, Psychology and Neurosciences, King's Col. London, London, United Kingdom; [4]Royal Society and Acad. of Med. Sci., London, United Kingdom

Abstract: Here we present our experimental and clinical findings, on the HPA axis function and its association with epilepsy and depression.
It is also known that dysregulation of the HPA axis might lead to stress-sensitive epilepsy. We tested the effects of stress on seizure susceptibility on the Wistar Audiogenic Rats (WAR), a

strain susceptible to sound-induced seizures, which show HPA axis hyperactivity. Adult WARs exposed to 1-hour of stress prior to each sound-induced seizure, in a 20-seizures protocol, expressed significant ($p<0.05$) higher seizure severity and lower latency for tonic seizure than non-stressed WARs. Administration of Gluco-and Mineralocorticoid receptors (GR and MR) antagonists, 0.5 hour before the stress, blocked the effects of stress.
Disturbance in the HPA axis is associated with worse outcomes, treatment-resistance, relapse and cognitive deficits in epileptic as well as depressive (MDD) and bipolar (BD) patients. Experimentally, we assessed early-life stress (ELS) effects on HPA axis function and its association with depressive-like behavior. Wistar rats were stressed from P1 to P21 or kept undisturbed; plasma corticosterone levels were significantly ($p<0.01$) higher in stressed rats than in controls at P3, P14 and P21 indicating no habituation to the protocol. At P90, rats that underwent ELS showed an anhedonic profile identified by a significant ($p<0.05$) decrease in sucrose consumption.
Clinically, patients were diagnosed with BD (n=18) or MDD (n=8) through the International Neuropsychiatric Interview; scores ≥16 in Hamilton Depression Rating Scale were included. When these patients, were submitted to HPA axis challenges with placebo, MR agonist and MR antagonist, administrated at 22:00, their plasma cortisol response, at 09:00 (next day), did not differ from healthy subjects, regardless of patients' ELS history. Post-hoc analyses indicated that BD patients show significant ($p<0.05$) lower cortisol levels than MDD patients after Placebo or MR agonist administration. But cortisol from DB patients was significantly ($p<0.01$) higher after MR antagonist treatment than placebo. In the other hand, cortisol levels of MDD patients were significantly ($p<0.05$) lower after MR agonist treatment than Placebo.
Pre-clinical experiment mimicking these challenges, in a more controlled condition, did not detect effects of ELS on pharmacologically-induced HPA axis response later in life. Furthermore, the cortisol response cannot discriminate patients with or without ELS history but support differential psychiatry diagnose between MDD and BD. Moreover, MR dysregulation plays an important role into Bipolar Depressive physiopathology.

Disclosures: **E.H. Umeoka:** None. **C.W. Baes:** None. **L.D. Godoy:** None. **N.C.B. Barroca:** None. **M.S.S. Umeoka:** None. **J. Antunes-Rodrigues:** None. **N. Garcia-Cairasco:** None. **M.F. Juruena:** None.

Poster

631. Risk Factors for Brain Disorders

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Program#/Poster#: 631.08/CCC22

Topic: A.07. Developmental Disorders

Support: NICHD P50 HD055784

NIMH RO1 MH100028

Title: Additive genetic risk for Autism Spectrum Disorder relates to altered functional connectivity

Authors: *K. E. LAWRENCE[1], L. M. HERNANDEZ[1], J. D. RUDIE[1], S. Y. BOOKHEIMER[1], P. LEVITT[2], D. H. GESCHWIND[1], M. DAPRETTO[1];
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Abstract: Autism spectrum disorder (ASD) is a heritable neurodevelopmental disorder that is diagnosed on the basis of impairments in social communication, as well as the presence of repetitive behaviors and restricted interests. Recent findings suggest that the majority of ASD's heritability is explained by the cumulative influence of many common genetic variants, underscoring the importance of studying the impact of such variants on the brain. Prior studies have found that ASD-associated common genetic variants, or single nucleotide polymorphisms (SNPs), relate to decreased long-range connectivity as measured by diffusion tensor imaging and functional magnetic resonance imaging (fMRI) in humans. However, all ASD neuroimaging studies to date have focused on SNPs in single genes despite clear evidence that ASD is related to SNPs across a multitude of genes.

To address this gap in the literature, we investigated how SNPs across multiple genes cumulatively relate to functional connectivity in children and adolescents with and without ASD. Specifically, we created a linearly additive genetic risk score based on subjects' genotypes in a total of seven SNPs across three genes which have been consistently linked to ASD (*CNTNAP2*: rs7794745, rs2710102. *MET*: rs1858830. *OXTR*: rs2254298, rs237887, rs1042778, rs53576). We then related this additive risk score to resting-state functional connectivity as measured by fMRI within two seed-based networks previously found to display atypical connectivity in ASD: the default mode network (DMN) and the salience network (SN). Results were prethresholded in FSL with a mask of within-network connectivity and thresholded at $Z>2.0$, cluster-corrected for multiple comparisons ($p<.05$). The typically-developing (TD) group (n=31) and the ASD group (n=37) did not significantly differ in number of risk alleles, and there was no main effect of diagnosis or risk score on the potential confounds of age, IQ, or mean relative motion.

Within the DMN, higher genetic risk related to decreased connectivity of the medial prefrontal cortex (mPFC) in the ASD group, but increased angular gyrus connectivity in the TD group. Within the SN, greater aggregate risk was related to diminished connectivity of the mPFC in ASD, but greater connectivity of lateral and medial frontal regions in typical development. Our results suggest that common genetic variants associated with ASD additively impact the connectivity of networks related to social cognition (the DMN) and the identification of and response to salient stimuli (the SN). Furthermore, the relationship between genetic risk and connectivity may vary as a function of diagnosis.

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Poster

631. Risk Factors for Brain Disorders

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 631.09/CCC23

Topic: F.04. Stress and the Brain

Support: Fondo de Investigacion en Salud

Title: Early life stress induced effects on hippocampal neurogenesis and HPA axis are not related to metabolic risk in mature rats; implications in diabetes and depression comorbidity

Authors: R. RUIZ[1], E. PINEDA[1], A. ROQUE[1], *N. LAJUD[2];
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Abstract: Obesity, depression and aging have all been linked to abnormal functioning of the hypothalamic-pituitary-adrenal (HPA) axis. Furthermore, health problems associated with aging might cause both weight gain and depression or anxiety. Early environmental influences are emerging as contributors to the development of both depression and metabolic disease. In rodents, early-life stress (ELS) decreases hippocampal neurogenesis and causes a deregulation of the HPA axis. Periodic maternal separation (MS) is a rodent model of ELS that affects hippocampal neurogenesis and increases HPA axis activity, this alterations are related depressive like behavior and mild metabolic inbalance in juveniles; however, the interaction between MS and aging induced metabolic alterations has not been studied. Therefore the aim of the study was to test the hypothesis that MS increases age-induced effects on hippocampal neurogenesis and metabolic risk. To test this, Sprague Dawley male rats were subjected to MS during the first two weeks of life (3 hours/day form P1-P14). We evaluated depressive-like behavior in the FST and anxiety-like behavior in the elevated plus maze (EPM) at 10 months of age. Animals were catheterized in the jugular vein and let to recover for one week. We evaluated glucose tolerance and determined the concentration of glucose, triglycerides, cholesterol, CORT and insulin. Rats were intracardialy perfused, brains were dissected and we performed an immunostaining against the immature neurons marker doublecortin (DCX). We estimated the number and density of labeled cells by stereology. MS animals showed a passive coping strategy in the FST without affecting anxiety-like behavior in the EPM. The glucose tolerance test showed that both MS and CONT animals have impaired glucose regulation. We observed no significant differences when comparing other metabolic parameters. However, MS decreased the density (cel No/mm^3) and number of DCX+ cells in the hippocampal neurogenic niche, without causing differences in the volume. In conclusion, our results suggest that metabolic alterations induced by age did not correlate with the effects of MS on hippocampal neurogenesis.

Disclosures: R. Ruiz: None. **E. Pineda:** None. **A. Roque:** None. **N. Lajud:** None.

Poster

631. Risk Factors for Brain Disorders

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 631.10/CCC24

Topic: F.04. Stress and the Brain

Support: Bucknell University Research Funding

Title: Excess glucocorticoid exposure during early life lead to changes in serotonergic function and increased risk of mood disorder in the adult male

Authors: *M. J. BONESSI, K. C. PAGE;
Biol., Bucknell Univ., Lewisburg, PA

Abstract: It is now widely recognized that exposure to an adverse environment during prenatal development can have lasting effects on an individual's physiology and risk of disease. Maternal stress, and thus exposure to excess glucocorticoid during pregnancy, has been associated with emotional and cognitive deficits in early life. Given their potent actions during development, excess glucocorticoids may represent a common pathway by which poor environmental conditions are signaled from the mother to the fetus triggering persistent changes in the offspring's growth and organ function. Our study was designed to examine the impact of prenatal exposure to a clinical antenatal steroid, Dexamethasone (Dex), and its effects on behavior and cortical expression of key pre- and postsynaptic proteins of adult male rats. Pregnant rat dams were injected once daily with Dex (sc 150ug/kg/day) from gestation-day 14 through 19. Control dams were injected with vehicle. At 120 days, the adult male offspring were tested using the elevated-plus maze (EPM) and the light-dark box to evaluate anxiety-like behavior in both control and Dex-exposed animals. At 150 days, the frontal cortex was rapidly dissected and frozen using liquid nitrogen. Real-time PCR was used to measure the relative levels of mRNA expression for serotonin receptors 5HT1A, 5HT1B, and 5HT2A as well as for key presynaptic proteins involved in vesicle release, synaptotagmin and SNAP-25. Using the EPM, a significant difference was detected between the control and Dex-exposed adult males in the ratio of open arm entries to total arm entries ($p= 0.031$). A significant difference was also observed between the control and Dex- exposed males when the amount of time spent in the open arms was compared to total time on the apparatus ($p= 0.018$). The light-dark box test, which also measures anxiety-like behavior, revealed that Dex-treated animals were more likely to cross to the dark side ($p=0.05$). Moreover, a significant increase in cortical mRNA expression was detected for the serotonin receptors 5HT1A ($p \leq 0.05$) and 5HT2A ($p < 0.05$). The mRNA levels for the 5HT1B receptor, synaptotagmin and SNAP -25 were not significantly changed in the cortex of the Dex-exposed adult males compared to controls. These data support the claim that epigenetic changes during early development lead to genetic programming effects that

persist throughout life. More specifically, prenatal exposure to glucocorticoid during critical periods of brain development disturbs serotonergic function and increases the risk for mood disorders in adult male offspring.

Disclosures: M.J. Bonessi: None. **K.C. Page:** None.

Poster

631. Risk Factors for Brain Disorders

Location: Halls B-H

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Program#/Poster#: 631.11/CCC25

Topic: F.04. Stress and the Brain

Title: Is acute or chronic juvenile stress a risk factor for PTSD in adulthood?

Authors: *L. CHABY[1], I. LIBERZON[2];
[1]Psychiatry, Liberzon Lab., [2]Univ. of Michigan, Ann Arbor, MI

Abstract: Many factors are suggested to contribute to resilience and susceptibility to post-traumatic stress disorder (PTSD), a chronic, debilitating disorder that can develop after exposure to a traumatic event. Exposure to trauma in childhood has been identified as a key risk factor for PTSD, but animal studies suggest that exposure to stress during the juvenile life stage can both increase stress responsivity and enhance functioning under future stress. We tested the hypothesis that adult rats exposed to chronic stress during juvenile development exhibit exacerbated responses to an animal model of PTSD, single prolonged stress, compared to adult rats reared without stress. We evaluated the lasting effects of both acute and chronic stress in juvenile development on PTSD specific end-points. We report that juvenile stress exposure affects aspects of fear learning and memory, and discuss effects of juvenile stress on morphological traits.

Disclosures: L. Chaby: None. **I. Liberzon:** None.

Poster

631. Risk Factors for Brain Disorders

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Topic: A.07. Developmental Disorders

Support: NIH Grant U54 HD083211

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NIH MH102272

Marino Autism Research Institue

Wallace Foundation

Title: Neural correlates of sensory hyporesponsiveness in toddlers at high risk for autism spectrum disorder

Authors: *D. M. SIMON[1], C. R. DAMIANO[2], T. G. WOYNAROSKI[1], L. V. IBANEZ[3], M. MURIAS[2], W. L. STONE[3], M. T. WALLACE[1], C. J. CASCIO[1];
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Abstract: Altered patterns of sensory responsiveness are frequently reported in individuals with autism spectrum disorder (ASD), and are hypothesized to contribute to the development of higher order social and behavioral deficits. Younger siblings of individuals with ASD are at a greatly elevated risk of future diagnosis, and patterns of reduced sensory responsiveness have been shown to emerge early in their development. Previous research has not characterized how patterns of neural activity in these high risk children relate to profiles of sensory responsiveness. Using high density electroencephalography (EEG) we investigated the neural correlates of parent reported sensory responsiveness in 18 month old siblings of individuals with ASD. We found that sensory hyporesponsiveness was associated with relatively increased left frontal alpha power and absolute increased left frontal theta power. Functional connectivity was increased to right frontal regions, decreased to left frontal regions, and broadly elevated in temporal and occipital regions in participants with high levels of hyporesponsiveness. Complementary measures of EEG signal complexity indicated hyporesponsiveness was associated with less complex neural signals. Multiple measures of neural activity thus corresponded with sensory hyporesponsiveness

in toddlers at high risk for ASD. We conclude that concurrent measurement of neural signal features holds promise for early identification of profiles of sensory responsiveness in young children at risk for ASD and improved targeting of interventions.

Disclosures: **D.M. Simon:** None. **C.R. Damiano:** None. **T.G. Woynaroski:** None. **L.V. Ibanez:** None. **M. Murias:** None. **W.L. Stone:** None. **M.T. Wallace:** None. **C.J. Cascio:** None.

Poster

631. Risk Factors for Brain Disorders

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Program#/Poster#: 631.13/DDD1

Topic: F.04. Stress and the Brain

Support: DARPA grant W911NF1010093

Title: Sphingosine 1 phosphate 3 receptors in the medial prefrontal cortex promote resilience to social defeat

Authors: ***B. CORBETT**[1], N. SOTUYO[2], J. PEARSON-LEARY[2], S. LUZ[2], S. BHATNAGAR[2];
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Abstract: Repeated exposure to stress promotes depressive- and anxiety-like behaviors in animals and the development of psychiatric disorders in humans, including depression and posttraumatic stress disorder. However, not all stressed individuals go on to develop psychiatric disorders. That is, some individuals are resilient to the effects of stress and others are vulnerable to its effects. In a paradigm of chronic social defeat in rats, we have identified subpopulations that are resilient or vulnerable to the neuroendocrine, prodepressive, and anxiogenic effects of defeat. Rats that exhibit a short defeat latency (SL) following placement in the cage of an aggressive resident rat are vulnerable to the effects of stress whereas rats that exhibit longer defeat latencies (LL) are resilient to the effects of stress. SL rats display increased depressive-like and anxiety-like behaviors compared to LL rats. However, the mechanisms underlying stress resilience and vulnerability remain unclear. We used a targeted PCR array approach to identify novel neural substrates underlying resilience and vulnerability. We found that the expression of sphingosine-1-phosphate receptor 3 (S1PR3) was increased in the medial prefrontal cortex (mPFC) of LL rats. S1PR3 is a G-protein-coupled receptor (GPCR) that reduces inflammation and, as an S1PR, has the ability to reduce neuronal activity through G_i coupling and activation of G-protein-coupled inwardly rectifying potassium channels. The mPFC regulates the stress response and affective-like behaviors, is highly susceptible to stress, and is dysfunctional in

stress-related affective disorders. We used a viral vector to overexpress S1PR3 in the mPFC of rats and found that S1PR3 overexpression increased defeat latency and decreased depressive-like and anxiety-like behavior compared to controls. Conversely, virally mediated S1PR3 knockdown decreased defeat latency and increased depressive-like behavior. Similar to LL rats, rats overexpressing S1PR3 in the mPFC displayed facilitated adrenocorticotropic hormone (ACTH) production during restraint compared to controls. S1PR3 knockdown rats displayed attenuated ACTH production during restraint compared to controls. Preliminary results indicate that pharmacological activation or overexpression of S1PR3 in the mPFC decreased neuronal activity and inflammation markers. We propose that S1PR3s in the mPFC promote resilience to stress by altering activity in the mPFC and within its extended network and/or by decreasing inflammation. We conclude that the S1PR3 is a novel receptor that promotes behaviors and neuroendocrine responses characteristic of resilient individuals.

Disclosures: **B. Corbett:** None. **N. Sotuyo:** None. **J. Pearson-Leary:** None. **S. Luz:** None. **S. Bhatnagar:** None.

Poster

631. Risk Factors for Brain Disorders

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Program#/Poster#: 631.14/DDD2

Topic: F.04. Stress and the Brain

Support: NIH Grant 2R15MH093918-02

Title: Early life sleep restriction induces behavioral and cognitive impairments in Sprauge Dawely rats

Authors: *F. ATROOZ[1], H. LIU[2], S. SALIM[2];
[1]Pharmacol. and Pharmaceut. Sci., [2]Pharmacol. and Pharmaceut. sciences, Univ. of Houston, Houston, TX

Abstract: Adequate sleep during early life is considered essential for normal brain function. In fact, sleep loss or sleep disturbances in children are associated with development of later life mood disorders. Thus, while negative effects of early life sleep deprivation are well known, information regarding repeated sleep restriction (RSR) during early life (childhood and adolescence) on behavioral and cognitive function is limited. Also, information regarding mechanisms by which RSR during early life causes behavioral and cognitive impairments are also not fully understood. In this study, two groups of Sprague Dawley rats (12 per group) were utilized, control group and RSR group. Rats, at postnatal day (PND) 18 were subjected to RSR

for 14 days (6 hours/day) using Pinnacle automated sleep deprivation system. This apparatus is a cylindrical cage with rotating arms that gently disturb the rats constantly interrupting their sleep. Behavioral tests (anxiety-like behavior, depression-like behavior) and cognitive tests (short-term and long-term learning memory function) were performed at PND 32, 60 and 90. Our results show that rats in the RSR group exhibited anxiety like behavior at PND 32 and 60 but not at PND 90 time point, as compared to the control group. Interestingly, RSR rats did not exhibit depression-like behavior at PND 30 or 60 but developed depression-like behavior at PND 90, as indicated by increased immobility time in forced swim test compared to control group. Furthermore, rats in the RSR group showed learning impairment at PND 60 as revealed by radial arm water maze test. On the other hand, RSR rats showed no impairment in short or long term memory at any time point. In conclusion, early life repeated sleep restriction promotes anxiety-like behavior early in life (PND 30 and 60) which in later life transforms into depression-like behavior (PND 90). Cognitive impairment also was evident at PND 60 suggesting later onset of cognitive dysfunction in Sprague-Dawley rats.

Disclosures: **F. Atrooz:** None. **H. Liu:** None. **S. Salim:** None.

Poster

631. Risk Factors for Brain Disorders

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Program#/Poster#: 631.15/DDD3

Topic: F.04. Stress and the Brain

Support: ARC DP150104835

NHMRC APP1031688

Title: A face only a mother could love: intergenerational trauma exposure reduces male attractiveness to potential mates.

Authors: *S. ALTMANN, J. M. KAN, R. RICHARDSON;
Psychology, The Univ. of New South Wales, Unsw Sydney, Australia

Abstract: The fact that some members of our species are more attractive to the opposite sex than others is well known. However, other than the obvious candidates such as genetics, the factors that determine “attractiveness” remain elusive. The current research used a rodent model to examine this generally understudied, but clinically relevant question, and assessed whether exposure to trauma, either within one’s one life, or intergenerationally, can impact male attractiveness in adulthood. To assess this question male rats were either exposed to Maternal

Separation (MS), a well-validated early life stressor during infancy, or were born to mothers who had experienced MS with their previous litter. Control females were then tested in a mate preference task, wherein she was given 4 trials in which she could choose between two adult males contained within small wire cages, either a control male and a directly-stressed male, or a control male and a male born to a previously stressed mother. The results showed that while females do not discriminate between control males and directly-stressed males, they do discriminate between control males and males born to a previously stressed mother, spending significantly less time across trials in the side of the test arena containing the male born to a previously-stressed mother, and investigating the wire cage in which that male was held significantly less. These results show that an individual's intergenerational exposure to stress may be even more important than their own history of trauma in determining social outcomes such as the ability to attract a mate.

Disclosures: S. Altmann: None. **J.M. Kan:** None. **R. Richardson:** None.

Poster

631. Risk Factors for Brain Disorders

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Topic: F.04. Stress and the Brain

Support: UC Davis Academic Federation Innovative Development Award

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CNPRC base grant P51 OD0011107

Title: Paternal line transgenerational effects of early experiences.

Authors: *E. L. KINNALLY, S. J. MARTINEZ, J. P. CAPITANIO;
Psychology, UC Davis, Davis, CA

Abstract: It has recently become clear that the effects of early life experiences may not be limited to the exposed generation, but can sometimes influence mental and physical health in the next generation. The mechanisms of transmission are often social in nature, but recent evidence suggests that germ line mechanisms may play a role. We compared the transgenerational effects

of maternal and paternal line early life nursery rearing (NR) on anxiety and health-related traits in two generations of rhesus macaques, including infants that did not have social access to parents because they were cross-fostered to new mothers and social groups. Offspring and grand-offspring (N=340) of NR and CONTROL reared individuals were observed for temperamental nervousness, immune cell counts, and plasma cortisol response to challenge. Paternal NR was associated with greater nervousness and lower immune cell counts in male and female infants ($F(3, 316) = 3.906$, $p = .009$, partial eta2 = .036). This effect persisted into the third generation through the paternal line ($F(3, 316) = 6.298$, $p < .001$, partial eta2 = .056). Maternal-line NR effects were not observed. Germ line mechanisms may be involved, as paternal NR effects were observed in the absence of social contact between parents and cross-fostered offspring. This study suggests that stress-related traits may be "inherited" across generations in primates, and points to non-social mechanisms of inheritance.

Disclosures: E.L. Kinnally: None. **S.J. Martinez:** None. **J.P. Capitanio:** None.

Poster

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Topic: F.04. Stress and the Brain

Support: R01-MH103102

R01-MH100894

Title: Iterative somatic transmission of emotional behavioral abnormalities across generations

Authors: *R. J. CHEN[1], E. MITCHELL[2], S. L. KLEIN[2], J. GAL TOTH[2], P. BERGIN[2], M. TOTH[2];
[1]Neurosci. Grad. Program, Weill Cornell Grad. Sch. of Med. Sci., New York, NY; [2]Dept. of Pharmacol., Weill Cornell Med. Col., New York, NY

Abstract: Though inheritance has traditionally been associated with the Mendelian transmission of genetic information from parents to offspring in allele-dependent manners, it has been shown that progeny can inherit parental behavioral traits through non-genetic mechanisms. While gametic transmission is accepted as a non-Mendelian means of multigenerational inheritance, we show that there is a somatic maternal mechanism for propagation of behavioral abnormalities that is maintained by an iterative process across generations. Studies show that infection and chronic low-grade inflammation during pregnancy increase the risk of psychiatric disorders in

the offspring. Maternal immune dysregulation may be transmitted to the fetus through the placenta past the blood brain barrier, and we present evidence suggesting the maintenance of a multigenerational transmission of abnormal emotional behaviors through a maternal immunological mechanism arising from a deficit in serotonin 1A receptor (5HT1AR). Specifically, our 5HT1AR heterozygote and null mice show increased innate anxiety and reduced motivation, and we show that these behavioral traits are passed up to the F3 offspring iteratively, one generation at a time, through non-gametic means. 5HT1AR is expressed on hematopoietic cells, and so we assessed the immune profiles of multiple generations of offspring and show alterations in their immune profiles. Right after birth, 5HT1AR-deficient mice and their F1 and F2 offspring all exhibit neutrophilia and monocytosis. Furthermore, this peripheral inflammation is accompanied by neutrophil/monocyte transmigration into brain parenchyma, suggesting brain immune activation. We believe brain and peripheral inflammation together explain the behavioral phenotype and accompanying neuronal epigenetic changes observed in adulthood. Also consistent with early life immune activation is the later developing autoimmune-like phenotype, characterized by increased total IgG level, anemia, lymphyocytopenia, and thrombocytopenia. Since maternal autoimmunity increases the risk for offspring immune activation and psychopathology, such mechanisms may explain the iterative nature of mother-to-offspring behavioral transmission. These results suggest that 5HT1AR may play an integral role in immune homeostasis, and that lower than normal 5HT1AR levels seen in a population of anxiety and depression patients may contribute to the psychopathology via low-grade sterile inflammations. Furthermore, our work here shows that multigenerational transmission is not limited to gametic mechanisms, but can occur iteratively through a somatic mechanism.

Disclosures: **R.J. Chen:** None. **E. Mitchell:** None. **S.L. Klein:** None. **J. Gal Toth:** None. **P. Bergin:** None. **M. Toth:** None.

Poster

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Topic: F.04. Stress and the Brain

Support: NIH Grant NIEHS 1RO1-ES022030

Title: Paraquat exposure *In utero* alters gene expression patterns in neonate c57bl6 pup microglia and astrocytes post exposure

Authors: *C. WALDEN, S. PANDEY, P. W. HALCROW, D. GUO, A. M. FLODEN, J. E. OHM;
Univ. of North Dakota, Grand Forks, ND

Abstract: Neural disease is a prevalent upcoming concern with the age of onset fast approaching in the population of the United States. The etiology of many neural diseases is unclear and may have links to environmental influences. Epidemiological studies indicate a higher risk for brain cancers for children of pesticide exposed parents and increased risk of neurodegenerative disease in adults residing in high exposure regions. Similar to the Barker Hypothesis we propose that early stress events lead to lasting epigenetic changes that predispose an individual to later onset of disease. To evaluate the impact of stressors in early development we utilized an in utero exposure model of c57bl6 mice exposed to the herbicide paraquat, or control saline, for six weeks over the gestational period. Paraquat is a model toxicant in the study of parkinsonian like symptoms in c57bl6 mice and is known to produce reactive oxygen species through inhibition of mitochondrial function. Adult mice were evaluated for impact of paraquat with histological staining of glial fibrillary acidic protein (GFAP) to measure reactive gliosis. Adult mice treated with paraquat exhibit marked increases in the levels of GFAP compared to controls indicating a reactive response to paraquat treatment. Neonatal pups were collected and astrocytes and microglia were cultured for two weeks removed from paraquat exposure, at which point RNA and DNA were collected from each respective population. RNA sequencing determined a limited number of genes that generally increase in gene expression. Ontological analysis indicate cell to cell interactions as well as developmental processes in these cells are largely impacted. The results of this study indicate that the exposure of mothers to paraquat provides an altered in utero environment changing gene expression in a persistent manner in the offspring. These changes may result in long term ramifications in the function and onset of disease in the central nervous system.

Disclosures: C. Walden: None. **S. Pandey:** None. **P.W. Halcrow:** None. **D. Guo:** None. **A.M. Floden:** None. **J.E. Ohm:** None.

Poster

631. Risk Factors for Brain Disorders

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 631.19/DDD7

Topic: F.04. Stress and the Brain

Support: Support from Shota Rustaveli National Science Association

Title: Revealing of hormetic dose of ethanol for white rats

Authors: *L. GOBECHIA-DAVLIANIDZE;
I.Beritashvili Ctr. of Exptl. Biomedicine, Tbilisi, Georgia

Abstract: Hormesis is a known biological phenomenon by which the body reaches a biologically positive results after the action of stress factors of different nature. It is known that receiving low doses of ethanol in human being is associated with positive events - reduced risk of cardiovascular disease, slowing the aging process and others. The aim of our study was to identify hormetic dose of ethanol for pregnant rats and after that to conduct research on their offsprings - whether or not they manifest any improvement or disorders in the processes of learning and memory (compared with the control animals). In the first series of experiments, testing of intact females held in the multi-way maze. The number of committed errors and time spent on the passage of the maze were registered. Within a 7-day testing sessions all animals reached an automatism in maze passage). Thereafter, the female rat together with male for 5 days was placed in the box. Then on already pregnant rat, we try to reveal the hormetic dose of ethanol - 21 days the rats injected (i/p) with different doses of ethanol (95% alcohol in doses ranging from 0.01 to 0.1 ml /100g). Each doses were tested on 5 rats. In all cases (the ethanol was diluted in 1 ml of the distillate). Performing of maze test was checked in 30 minutes after the administration of ethanol. The most pronounced behavioral manifestation of hormetic effect (acceleration of motility) was revealed at a dose of 0.025 mg / 100g. Administration of 1 ml saline (control animals) caused no change in performing of maze test. Thus it can be said that the hormetic effect of ethanol on rats may be observed at a dose of 0.025 mg / 100g. In the next series of experiments we want to clarify if administration of hormetic dose of ethanol during the pregnancy will cause any physiological (including learning and memory processes and/or morphological changes in offsprings. **Acknowledgement:** The study was supported by the Shota Rustaveli National Science Foundation.

Disclosures: L. Gobechia-Davlianidze: None.

Poster

631. Risk Factors for Brain Disorders

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Program#/Poster#: 631.20/DDD8

Topic: F.04. Stress and the Brain

Support: CONACyT Grant 221092

CONACyT Grant 238313

Title: Activation of proto-oncogene c-fos in non-auditory structures of the rats´ brain under stimulation with environmental noise

Authors: D. FERNANDEZ-QUEZADA[1], D. MORAN-TORRES[1], S. LUQUIN[1], J. GARCÍA-ESTRADA[2], Y. RUVALCABA-DELGADILLO[1], *J. H. FERNANDO[1];
[1]Univ. de Guadalajara, Guadalajara, Mexico; [2]Ctr. de Investigación Biomédica de Occidente, Guadalajara, Mexico

Abstract: Backgroung: Noise is an inarticulate auditive stimulus that often becomes annoying. Pollution by environmental noise has increased in parallel with development of human activities (airway, railway, roads and the traffic). In general, the effects of noise on central nervous system may be classified as auditory and non-auditory. Most of the non auditory effects involve the stress HHA regulatory system. Then, it is expected that central structures mediating stress responses could change its activity patterns under noise exposure. Objective: In this experiment, we exposed adult male rats to a noisy environment and immunohistochemically evaluated c-fos changes over the main central structures mediatin stress response. **Material and methods:** 25 male wistar rats were subjected to a rats´ audiogram-fitted adaptation of a noisy environment and sacrificed at different time points as follows: 0 (n=5), 2h (n=5), 6h (n=5), 12h (n=5) and 24h (n=5). Immunohistochemistry against c-fos was performed on 70µm slices belonging to the entire brain. **Results:** We found differential patterns of c-fos expression depending on region analyzed and time of exposure. Then, our results support the idea that noise exposure produce stress-like effects by activating some non-auditory structures involved in stress regulation.

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Poster

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Topic: F.04. Stress and the Brain

Support: This work was supported by the War Related Illness and Injury Study Center within the Department of Veteran Affairs.

Title: Post traumatic stress disorder is not associated with increased sympathetic activity in US veterans

Authors: *Y. HABER[1,2,4], K. MIGDAL[4], K. BREWER[4], J. SERRADOR[3,4], H. CHANDLER[4]; [1]Newark, NJ; [3]Physiol. and Neurosci., [2]Rutgers Univ., Newark, NJ; [4]War Related Illness and Injury Study Ctr., East Orange, NJ

Abstract: Posttraumatic stress disorder (PTSD) is defined as an anxiety disorder provoked by an event that evokes extreme fear, helplessness, or horror such as an accident, physical or emotional abuse or combat. The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) identifies three clusters of symptoms that comprise PTSD: intrusive thoughts and re-experiencing of the trauma in dreams and nightmares, avoidance of circumstances of the trauma and a persistent hyperarousal state often including hypervigilance and an exaggerated startle response. It has been assumed that due to hyperarousal contributes to increased sympathetic activity in patients with PTSD. This makes logical sense since sympathetic activity is associated with a fight or flight response. However there is little data that has directly examined sympathetic activity in this population. The goal of this work was to determine if peripheral sympathetic activity was elevated in a group of Veterans with and without PTSD. Thirty Four US Veterans that were participating in studies to examine their autonomic function were classified as having PTSD by a score of 37 or greater on the PCL. Based on this 20 of the 34 would be classified as having PTSD. Non-invasive measures of sympathetic activity were obtained from beat by beat heart rate and blood pressure variance as well as heart rate and blood pressure. There was no difference in resting heart rate (Ctrl: 72.0±11.2 vs PTSD: 70.2±9.7 bpm) or mean arterial blood pressure (Ctrl: 95.4±12.8 vs PTSD: 93.7±10.6 mmHg). Examining heart rate variance there was no difference in low frequency normalized power, indicative of cardiac sympathetic activity (Ctrl: 51.4±25.4 vs PTSD: 56.2±24.0%). Similarly there was no difference in low frequency blood pressure power, indicative of peripheral vascular sympathetic activity (Ctrl: 61.9±33.6 vs PTSD: 54.4±34.9 $mmHg^2$). Performing linear regressions there were no significant correlation between PCL score and any of the parameters. These data suggest that Veterans with PTSD may not demonstrate increased sympathetic activity. However, this was done on a small group of Veterans so confirming in a larger group would be essential. We also plan to look at whether Veterans who specifically demonstrate symptoms of hyperarousal tend to have increased sympathetic activity compared to those with avoidance characteristics. This work was supported by the War Related Illness and Injury Study Center within the Department of Veteran Affairs.

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Poster

632. Blood Flow and Functional Imaging

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: The Research Council of Norway (RCN) through its user-driven research (BIA) funding scheme, project number 236739/O30

Title: Autonomous Driving - measuring cognitive load via functional near infrared spectroscopy (fNIRS) for complex simulated and in-situ driving scenarios

Authors: *S. BALTERS[1], S. SIBI[2], J. WENDY[2], M. STEINERT[1];
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Abstract: Grounded and guided by the fundamentals in affective neuroscience and framed by the human-computer interaction (HCI) paradigm of affective computing, we call for the emergence of affective engineering as a key contributor to future product and system design and development. In an engineering context, we aim to quantitatively measure situational variables that allow us to control for (emotional) behavior responses; that is, we seek to use physiology sensors to "read" the human in critical human-machine interaction scenarios. With the particular focus on (simulated) autonomous driving scenarios, the Center for Design Research at Stanford University and the Department of Engineering Design and Materials at the Norwegian University of Science and Technology aim to address this challenge in a series of joint experiments. As the number of automation features in a car rises, the potential need for the driver to rapidly assess and resume control of the car increases. Simultaneously, the possibility for the driver to pursue secondary activities also emerges. Thus, in order to safely transfer control to the driver, it is vital to incorporate the driver's attentional, physiological, and mental state - one potential way is by using physiology sensors. In a first experiment, we use functional near infrared spectroscopy (fNIRS) in order to measure cognitive load during simulated autonomous driving including simple lane change maneuvers. The main arising challenge is to select/develop a suitable mathematical model for measuring cognitive load for in situ "chaotic" and "noisy" driving scenarios. Since the experimental task differs tremendously from clean laboratory experiments with highly isolated and controlled tasks (such as finger tapping or n-back task), applying proposed cognitive load algorithms that assume a constant response at trigger onset, such as statistical parametric mapping (SPM), are questionable. The ultimate aim is to build the scientific foundation for in situ cognitive load measures in order to design and engineer safe driving in the future.

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Poster

632. Blood Flow and Functional Imaging

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Program#/Poster#: 632.02/DDD11

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Effects of intraperitoneal injections of thc on bold signal using functional magnetic resonance imaging in awake rats

Authors: *D. MADULARU[1], P. KULKARNI[2], C. F. FERRIS[2];
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Abstract: Cannabis is one of the most frequently used illicit drugs worldwide, and has been banned in the United States and Canada since the early 1900s. A recent United Nations report places cannabis at the top of the 2010 illicit drug use list, at an estimated prevalence of 2-5% (120-220 million users), followed by opioids (0.8%), opiates (0.5%) and cocaine (0.4%). Although it has been suggested that the marijuana plant includes over 400 chemical entities of which 60 are cannabinoids, the most studied are Δ-9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD) and cannabinol (CBN); Δ9-THC has been identified to be the main psychoactive agent in cannabis. In addition to the recreational use, cannabis has been widely used to address a series medical conditions, through its anti-emetic, analgesic and appetite-modulating effects.
The purpose of the present study is to assess the effects of three doses (0.1, 1 and 10 mg/kg) of THC on brain activation using fMRI in awake rats. Although more than 170 regions of interest have been assessed, emphasis has been placed on areas rich in CB1 receptors, such as the hippocampus, hypothalamus and prefrontal formations. Overall, the low dose shows increased BOLD activation in a number of areas (ventral and dorsal striatum, basal amygdala, ventral CA1 and ventral pallidum) compared to vehicle. Interestingly, the BOLD activation in response to the low dose was also increased compared to the medium and high doses, although in some regions it follows a U-shaped pattern (i.e. temporal cortex).
These findings offer insight into brain networks activated by THC, providing the first evidence of brain-wide “fingerprinting” of BOLD activity in response to different doses of THC. The results are discussed in the context of pain networks and beyond.

Disclosures: **D. Madularu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PK and CF have financial interest in Animal Imaging Research and Ekam Imaging.. **P. Kulkarni:** None. **C.F. Ferris:** None.

Poster

632. Blood Flow and Functional Imaging

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Program#/Poster#: 632.03/DDD12

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: IBS-R015-D1 in Republic of Korea

Title: Hemodynamic alteration of cerebral cortex of mouse after chronic cranial window surgery

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Abstract: In brain imaging study, cranial window surgical processes are necessary to get an access to the animal's brain. The invasive surgical procedures would evoke neuroinflammatory mechanism. In particular, drilling and opening skull bone can induce mild traumatic brain injury. Our understanding of how invasive cranial window surgery affects neuronal and hemodynamic responses is very limited. Using cranial window with the soft, flexible, transparent, and biocompatible silicone-based polydimethylsiloxane (PDMS) (Heo et al, 2016, submitted), we investigated the effect of cranial window surgergical procedure on cerebral hemodynamics and its' timecourse. C57bl/6 mice were utilized. Following 2 hours, 14 days, 28days, 32days of surgery, we recorded cerebral blood volume (CBV) changes by whisker stimulation (C2 area) with the intrinsic signal optical imaging. Also we measured cerebral blood flow (CBF) with Laser Doppler Flowmetry. We found that the animals immediately after 2 hours of cranial window surgery have smaller CBV changes than the animals 14days, 28days, 32days post cranial window surgery. The maximum CBV changes was the largest in animals 32days post cranial window surgery. In immunohistochemical studies, we found that the expression level of GFAP of astrocyte and microglial activation is different among the animals of each group. Our study suggests that the cranial window surgical procedure can significantly affect the integrity of neuronal network even though no apparent cellular damages were found. In order to get normal brain hemodynamics, we need to wait at least 28 days post cranial window surgery.

Disclosures: **H. Park:** None. **C. Heo:** None. **N. Yu:** None. **M. Suh:** None.

Poster

632. Blood Flow and Functional Imaging

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Program#/Poster#: 632.04/DDD13

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Rapid changes in spontaneous neural activity are associated with hemodynamic fluctuations during different cortical states

Authors: M. BRUYNS-HAYLETT[1], R. SLACK[2], P. PATEL[2], B. GOUDE[2], S. HARRIS[2], L. BOORMAN[2], J. BERWICK[2], *M. JONES[2];
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Abstract: Spontaneous fluctuations in Blood Oxygen Level Dependent (BOLD) functional Magnetic Resonance Imaging (MRI) signals are used to infer functional connectivity. However, the implicit assumption that spontaneous fluctuations in blood oxygenation are related to the underlying spontaneous activity has only been investigated in a small number of studies. For instance, we previously made concurrent 2-dimensional optical imaging spectroscopy (2D-OIS) measurements of spontaneous cortical hemodynamics and multi-laminar electrophysiological measurements of spontaneous cortical activity in urethane anaesthetised rodents (Bruyns-Haylett et al., 2013). We demonstrated that rapid changes in spontaneous activity were associated with hemodynamics that were similar to those elicited by sensory stimuli thus suggesting an association between spontaneous activity and hemodynamic fluctuations. However, it is well known that rodents anaesthetised with urethane move between different states of anaesthetic depth (e.g. Friedberg et al., 1999) which are commensurate with states of quiescence and arousal in awake behaving animals (e.g. Castro Alamancos 2004). Previous studies have suggested that stimulus evoked neurovascular responses may differ in these different states (Jones et al., 2008) but as Bruyns-Haylett et al., (2013) neglected to classify data into these states, whether 'spontaneous' neurovascular responses are preserved during different cortical states is uncertain. Thus information that could aid the interpretation of resting state data in different states of wakefulness was lost. Fortuitously, a recent analysis methodology developed in our laboratory (Slack et al., 2016) allows such data to be sorted into 2 different 'anaesthetics depths' or 'cortical states' based on ongoing fluctuations in LFP. As such, the current investigation takes similar concurrent measurements of cortical hemodynamics and ongoing activity as Bruyns-Haylett et al., (2013) but classifies data into different cortical states before investigating the relationship between rapid spontaneous activity and the accompanying hemodynamics. As previously reported, we find that changes in cortical state themselves are accompanied by slow increases or decreases in total haemoglobin concentration and blood oxygen saturation (Jones et al., 2008, Slack et al., 2016). In both cortical states rapid increases in spontaneous activity are

accompanied by hemodynamics resembling those elicited by sensory stimuli suggesting that spontaneous activity elicits hemodynmic changes despite differences in wakefulness.

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Poster

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Program#/Poster#: 632.05/DDD14

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: FWF-Projekt P28261

Title: Sex differences in inter-hemispheric connectivity during global-local processing

Authors: ***T. HARRIS**, B. PLETZER;
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Abstract: Global-local processing is traditionally studied with hierarchical stimuli, e.g. a large global letter made up of small local letters (Navon paradigm). Participants are asked to identify targets either at any level (divided attention condition) or at a pre-specified level (selected attention condition). Performance is characterized by faster responses to global than to local targets (global advantage effect). Previous studies demonstrated a right-lateralization for global and left-lateralization for local targets. It is presumed that lateralization of brain functions are related to the inhibitory influence of a dominant on the non-dominant hemisphere. However, inter-hemispheric connectivity (IC) has previously not been investigated for global-local processing. Furthermore, we were previously able to demonstrate sex differences in the global advantage effect (stronger global advantage in men during selected attention) and the lateralization of BOLD-response to global and local level (stronger lateralization in women). Whether these differences result from sex differences in IC has not been investigated. 86 men and 41 naturally cycling women in their luteal phase completed a Navon paradigm, modulating attention condition (divided vs. selected) during functional MRI. Most prominent activations to both global and local level were observed bilaterally in the occipital lobe with stronger left lateralization for local and stronger right lateralization for global targets. The individual subject maxima were selected as ROIs for psycho-physiologic interaction (PPI) analyses with ROIs for analyses of global targets in the right-hemisphere and ROIs for analysis of local targets in the left hemisphere. In the divided attention condition, the results demonstrate negative IC of the right occipital lobe to the left occipital lobe for global targets. For local targets, a negative intra-

hemispheric connectivity from occipital to parietal areas was observed within the left hemisphere. These results were not modulated by sex. For the selected attention condition, however, negative IC from right to left occipital areas was only confirmed in men. Women, on the other hand, showed stronger intra-hemispheric connectivity with more anterior areas. For the first time, the global advantage effect during global-local processing and right-lateralization for global targets is demonstrated and may arise from the inhibitory influence of the right on the left hemisphere. Furthermore, sex differences in the global advantage effect and lateralization patterns during global-local processing may arise from sex differences in these IC patterns.

Disclosures: **T. Harris:** 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; FWF-Projekt P28261. **B. Pletzer:** 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; FWF-Projekt P28261.

Poster

632. Blood Flow and Functional Imaging

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Program#/Poster#: 632.06/EEE1

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Carle Neuroscience Inst.

Title: Effect of temperature on FAD and NADH-derived signals and neurometabolic coupling in brain slices

Authors: *B. A. IBRAHIM[1,2], H. WANG[1,2], B. BUCCI[1,2], K. PAUL[1,2], D. A. LLANO[1,2]; [1]Beckman Inst., Urbana, IL; [2]Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Therapeutic hypothermia has long been used as a method to protect the brain from ischemic insults, though the impact of temperature on brain metabolism and neurometabolic coupling is not well understood. Therefore, we examined the effect of temperature on brain metabolism using FAD and NADH auto-fluorescence metabolic signals and electrical field potentials from brain slices prepared from adult mice. We found that basal FAD signals are inversely proportional to temperature while basal NADH signals had a biphasic profile such that the signals decreased with increasing temperature from 13^{o}C to 21^{o}C (Phase I) then increased with increasing the temperature above 25^{o}C (Phase II). Different brain regions showed the same profile for FAD and NADH signals, but the hippocampus had the highest signal intensities for

both. Calculated redox ratio followed the FAD profile suggesting that the redox ratio changes with temperature are likely driven by FAD signals. In auditory thalamocortical slices, electrical stimulation of the subcortical white matter produced a biphasic profile with temperature; the magnitude of both FAD and NADH deviations from baseline were largest around 21-25^{o}C, and diminished at higher and lower temperatures. Additionally, SR95531 (gabazine) was used to elicit spontaneous paroxysmal events, and the link between field potentials and metabolic fluorescence signals was compared at different temperatures. Similar to the evoked responses, the magnitude of the spontaneous FAD and NADH signals adopted a biphasic profile with the temperature. Extracellular recording of spontaneous activity showed that local field potential signal amplitude was strongly coupled to FAD and NADH signal amplitude at temperatures from 13-25^{o}C, but deviations were seen at higher temperatures, such that field potential amplitude was retained to higher temperatures than the fluorescent metabolic signals. These results indicate the strong impact of temperature on brain metabolism which in turn affects neuronal activity and that neurometabolic coupling may show temperature dependence, which has potential implications for therapeutic modulation of brain temperature. In addition, the similarity between temperature-induced biphasic profile of NADH baseline and the metabolic signals coupled with evoked synaptic activity could possibly indicate that glycolysis and the TCA cycle are the most temperature affected metabolic processes.

Disclosures: **B.A. Ibrahim:** A. Employment/Salary (full or part-time): University of Illinois at Urbana-Champaign. **H. Wang:** A. Employment/Salary (full or part-time): Carle Foundation Hospital. **B. Bucci:** None. **K. Paul:** None. **D.A. Llano:** None.

Poster

632. Blood Flow and Functional Imaging

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Program#/Poster#: 632.07/EEE2

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH R01 NS095933

NIH R21 HL108143

Title: Characterization of the hemodynamic response function across human cerebral cortex

Authors: *J. KIM, A. TAYLOR, D. RESS;
Neurosci., Baylor Col. of Med., Houston, TX

Abstract: In functional magnetic resonance imaging (fMRI) experiments, use is made of the hemodynamic response function (HRF), the stereotypical vascular response evoked by brief (<4 s) neural activation. Use of the HRF to evaluate neurovascular function as a clinical tool is appealing, but there are two main challenges to such clinical applications. First, we need a method to evoke the HRF broadly across the brain, and measure the response specifically in parenchymal gray matter. Second, clinical use of the HRF will require a better understanding of its variability across brain regions and subjects. Here, we present a simple stimulus paradigm to evoke the HRF across the majority of cerebral cortex. The results provide guidance for linear analysis of fMRI data, and more importantly, create a potentially highly attractive clinical tool. **Methods**: A 2-s duration audiovisual stimulus was combined with a fast-paced task to evoke the HRF, and this was repeated ~85 times per session in 8 subjects with a 30-s inter-stimulus interval. FMRI (SMS excitation, 1.5-s/volume) with high spatial resolution (2-mm voxels) was used to focus measurements specifically on the gray matter. Our computational model was used to predict underlying CBF and $CMRO_2$ responses. **Results**: Mean amplitudes of the positive HRFs varied substantially across the cortical surface (Fig A), and from subject-to-subject. ~75% of cortex responded with significant HRFs. ~78% of active cortex exhibited positive HRFs (solid curves); the remainder showed inverted HRFs (dashed curves). However, after a parcellation and normalization procedure, the spatial pattern of the HRF amplitudes was found to be remarkably similar across subjects. The time-to-peak of the initial positive lobe of the HRF showed less subject-to-subject variability, and was relatively stable across the cortical surface (Fig B). **Conclusion**: This method provides a means to quantify neurovascular function across the majority of the brain, with great potential clinical utility to diagnose and monitor the treatment of vascular brain pathologies.

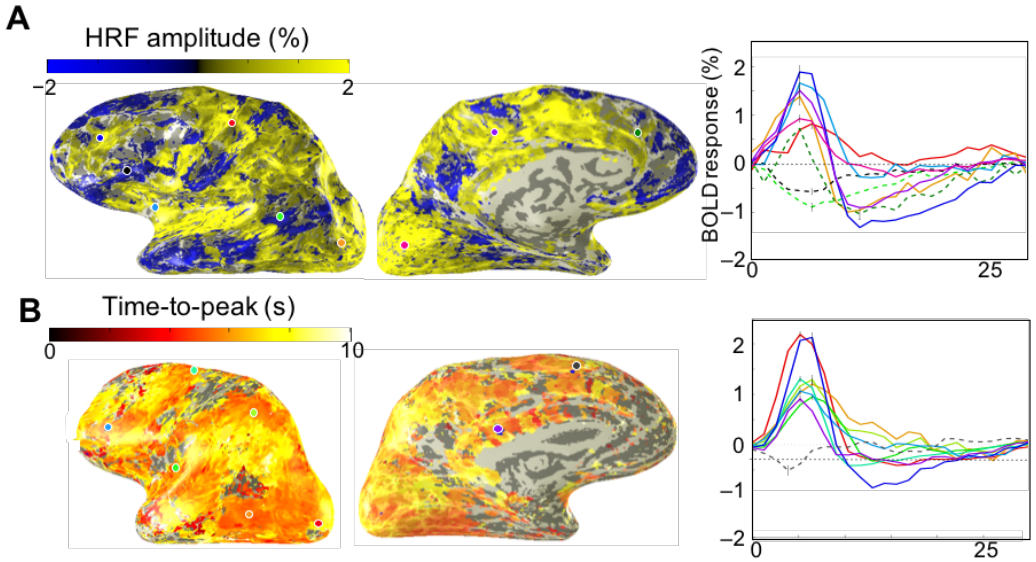

Figure: HRF parameters **A**) amplitude; B) time-to-peak. Plots on right show sample HRFs corresponding to color-coded dots on surface models

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Poster

632. Blood Flow and Functional Imaging

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Program#/Poster#: 632.08/EEE3

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Laterality Index plot of NIRS data indicates the brain activation laterality for calculation test and for Kraepelin performance test

Authors: *H. EDA[1], M. YAMAZAKI[2], N. OKAMOTO[3], Y. KURODA[4];
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Abstract: Introduction
Near infrared spectroscopy (NIRS) calculates hemoglobin parameters, such as changes in oxygenated hemoglobin (oxyHb) and deoxygenated hemoglobin (deoxyHb), assuming the modified Lambert-Beer's law. Laterality Index (LI) is one of the smart ideas for NIRS application to brain study. LI is calculated as (LT-RT) / (LT+RT), where LT is the activation of the left and RT Right. The LI has been popular for language research, measuring Broca's area. We measure forehead by NIRS system, and calculate Laterality indexes. The purpose of this study is to discuss the possibility of the LI plot of NIRS for cognitive research.
Methods
Subjects were required to perform 2 tests. One was a calculation test. Two numbers were appearing on the screen for 4 seconds, such as 62+59. The subject calculated, and typed the answer by ten-key. This was repeated for 50 times. The other was Kraepelin performance test for 5 minutes. We used the NIRS system (Spectratech, Inc., Japan) having 16 Source-Detector pairs (16 channels). The picture shows the area of 16 channels. Channel 1 to 7 were located on the right side, and channel 10 to 16 were located on the left side. We calculated seven Laterality indexes with oxyHb; LI1 (ch16-ch1), LI2 (ch14-ch2), LI3 (ch15-ch3), LI4 (ch13-ch4), LI5 (ch11-ch5), LI6 (ch12-ch6), LI7 (ch10-ch7). These indexes were plotted.
Results
LI plot of 2-digit calculation test and Kraepelin performance test were clearly different. For 2-digit calculation task, LI7 (ch10-ch7) was positive. For Kraepelin performance test, LI1 (ch16-ch1) was positive.
Conclusion
There are many reports on the forehead activation, using NIRS. But the discussion is confused, because we have few functional magnetic resonance images of frontal area. We calculated Laterality Indexes from NIRS multiple channel data. LI plot includes many indexes, and this clearly shows brain activation laterality. This plot can be applied to cognitive research. We know

the laterality pattern according the task and the pattern change before and after the task. This opens the new perspective of educational research.

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Poster

632. Blood Flow and Functional Imaging

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: ElectroCore, LLC (Basking Ridge, NJ)

Rutgers Institute for Data Science, Learning, and Applications - Rutgers University

Title: Cutaneous electrical stimulation of the neck accesses vagal projections: fMRI evidence in humans

Authors: *E. FRANGOS[1,2], B. R. KOMISARUK[1];
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Abstract: While direct stimulation of the vagus nerve via implanted electrodes is currently used as a treatment for refractory epilepsy and depression, recent developments in non-invasive approaches have demonstrated similar beneficial effects. The purpose of the present study was to ascertain whether afferent vagal projections can be accessed non-invasively by transcutaneous electrical stimulation of the antero-lateral surface of the neck, which overlies the course of the vagus nerve. Methods: Thirteen healthy subjects underwent 2 fMRI scans in one session. Each participant experienced transcutaneous electrical stimulation of the right postero-lateral surface of the neck during scan #1 (control condition, sternocleidomastoid stimulation: "SCM") and stimulation of the right antero-lateral surface of the neck during scan #2 (experimental condition, non-invasive vagus nerve stimulation: "nVNS"). The duration of stimulation was 2min for each condition, and was followed by a 15min post-stimulation period to assess possible persistent effects of the stimulation. Mean group effects were analyzed to determine whether nVNS activated classical vagal projections to the brainstem and forebrain, compared to baseline and SCM stimulation. Results: Compared to baseline and control (SCM) stimulation, nVNS significantly activated primary vagal projections including the nucleus of the solitary tract (primary central relay of vagal afferents), parabrachial area, primary sensory cortex, and insula. Regions of the basal ganglia and frontal cortex were also significantly activated. Deactivations were found in the hippocampus, visual cortex, and spinal trigeminal nucleus. During the post-nVNS period, there was initially regional deactivation, approximately 5min later some of the brain regions increased in activity (e.g., hypothalamus), followed by deactivation at the end of the post-nVNS period. Brainstem activations during the post-nVNS period were found within the substantia nigra, ventral tegmental area, raphe nuclei, and periaqueductal gray. Conclusion: The present findings provide evidence in humans that cervical vagal afferents can be accessed non-invasively via transcutaneous electrical stimulation of the antero-lateral surface of the neck (nVNS), which overlies the course of the nerve. These findings suggest an alternative and feasible method of stimulating vagal afferents.

Disclosures: **E. Frangos:** 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; ElectroCore, LLC (Basking Ridge, NJ, USA). C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Rutgers Institute for Data Science, Learning, and Applications - Rutgers University. **B.R. Komisaruk:** 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; ElectroCore, LLC (Basking Ridge, NJ). C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Rutgers Institute for Data Science, Learning, and Applications - Rutgers University.

Poster

632. Blood Flow and Functional Imaging

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IBS Grant R015-D1

Title: Vascular architecture with CLARITY suggests that contrast-enhanced high-resolution fMRI is dominated by microvessel dilation

Authors: *A. J. POPLAWSKY[1], H. FUKUDA[1], B.-M. KANG[2,3], J. KIM[2], K. CHUNG[5], M. SUH[2,3], S.-G. KIM[2,3,4];
[1]Radiology, Univ. of Pittsburgh, Pittsburgh, PA; [2]Ctr. for Neurosci. Imaging Res., Inst. for Basic Sci., Suwon, Korea, Republic of; [3]Biomed. Engin., [4]Biol. Sci., Sungkyunkwan Univ., Suwon, Korea, Republic of; [5]Chem. Engin., MIT, Cambridge, MA

Abstract: Introduction: Functional magnetic resonance imaging (fMRI) measures the hemodynamic response to neuronal activity, but more evidence is needed to understand how far the high-resolution fMRI response spreads relative to the evoked neuronal activity. The olfactory bulb is an ideal model system to study this issue because synapses localized to a single layer can be preferentially evoked by a selective stimulation. We previously showed that fMRI signal increases due to lateral olfactory tract (LOT) stimulation are highly localized to the evoked synapses in the external plexiform layer (EPL); but it is unknown whether dilation of local microvessels is the dominating source of our fMRI measurements.
Methods: In α-chloralose anesthetized rats, LOT was stimulated in a block design experiment (-200 μA, 200 μs pulse duration, 40 Hz, ~1 min stimulus duration, 4 min interstimulus interval); and high-resolution (55 x 55 x 500 μm^3) blood volume-weighted fMRI responses were measured at 9.4 T. Line profiles from 330-μm thick slabs that orthogonally transected the bulb layers were obtained and the full width at half maximum (FWHM) of the evoked fMRI peaks due to LOT stimulation were measured. In a different rat, blood vessels were stained with DyLight594 Tomato lectin (Vector Laboratories, DL-1177) and the anatomical vascular architecture of EPL

was imaged by 3D CLARITY. The FWHM data were compared to the vessel diameters, lengths and volumes calculated by volumetric analysis of the CLARITY images.
Results: The mean FWHM of the fMRI peaks was 347 ± 102 μm (mean ± SD, n = 30 peaks from 5 rats, 3 slices each rat, 2 peaks each slice), where the mean anatomical thickness of EPL at these lines was 265 ± 65 μm. The fMRI spatial spread beyond the anatomical thickness of EPL was estimated by a least squares linear regression analysis. The regression intercept (± SE), which approximates this spread, was 106 ± 65 μm ($r^2 = 0.34$, $p < 0.001$, $df = 28$). With CLARITY, microvessels that had diameters <12 μm accounted for the majority of the total vascular volume (65.8%) present in EPL and had an average length of 54.8 ± 41.2 μm (± SD, n = 398 vessel segments).
Conclusions: Our preliminary results indicate that the LOT-evoked fMRI signal spreads ~100 μm from the evoked layer EPL (~50 μm on each leg of the fMRI response peaks). This spread coincides with the mean length of microvessels (~50 μm), the predominate vascular compartment in EPL.

Disclosures: A.J. Poplawsky: None. **H. Fukuda:** None. **B. Kang:** None. **J. Kim:** None. **K. Chung:** None. **M. Suh:** None. **S. Kim:** None.

Poster

632. Blood Flow and Functional Imaging

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 632.11/EEE6

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Can we capture lateralization of brain activities with EEG and/or NIRS?

Authors: *M. YAMAZAKI[1], H. EDA[2], N. OKAMOTO[3], Y. KURODA[4];
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Abstract: Introduction
Near infrared spectroscopy (NIRS) calculates the changes in hemoglobin parameters. NIRS is widely used in brain science, psychology and clinical field to evaluate the brain activations because of its easy operation compared to other brain measurement systems. Purpose of this study is to validate the methods capturing the brain activities by simultaneous EEG and NIRS recording during Kraepelin test and 2-digit addition calculation task.
Methods
University students (average, 22 years old) from Mathematics Educational division were recruited as subjects. We conducted EEG and NIRS recording simultaneously during two types

of calculation tasks. One of the calculation tasks was Kraepelin test and the other was 2- digit addition test. NIRS : We used NIRS system (Spectratech, Inc., Japan) having 16 channels and put the sensors on the forehead. Laterality Indexes (LI) were calculated as (Left-Right) / (Left+Right) with the changes in oxygenated hemoglobin by NIRS system.EEG : We attached 10 scalp EEG electrodes (Fp1,Fp2, F3,F4,Fz,Cz,T3, T4, O1 and O2)on the head according to 10-20 system and recorded EEG data using polymate II AP216 (TEAC Corp., Japan). Time-frequency analysis was applied to EEG data to evaluate Fm theta wave activity.

Results

During the Kraepelin task EEG began showing Fm theta activities 40 seconds after started the task. These Fm theta activities showed maximum power (μV2) over the Fz electrodes and it also showed slightly higher power on the left side (F3) compared to the right side (F4). NIRS showed oxyHb elevation immediately after started the task. NIRS LI on lateral frontal channel which corresponded with F3 EEG position showed always positive. During the 2-digit addition task EEG showed no clear tendency however subtraction of theta band power by time frequency analysis showed obvious left predominant activities. All of NIRS LIs showed always positive.

Conclusion

This study showed that there were difference of the brain activities between 2-digit calculation task and Kraepelin task. These differences may need to be considered in the educational research. Combined with several measuring modalities give us additional information because the timing of the hemodynamic activation in NIRS differs from the neural activities in EEG.

Disclosures: **M. Yamazaki:** None. **H. Eda:** None. **N. Okamoto:** None. **Y. Kuroda:** None.

Poster

632. Blood Flow and Functional Imaging

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 632.12/EEE7

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Portal vein shunts in c57bl/6j mice and strains derived from it - serious implications to neuroscience research.

Authors: *K. LEHTIMÄKI, J. PUOLIVÄLI, T. HUHTALA, T. HEIKKINEN, A. SHATILLO, A. NURMI, P. SWEENEY;
Charles River Discovery, Kuopio, Finland

Abstract: Here we report our findings on portal vein shunts (PS) in naïve C57Bl/6J mice. This confirms and extends the study by Cudalbu et al. 2013 which showed that large portion of C57Bl/6J mice and strains derived from it suffer from this devastating anatomical defect. While

this anomaly must have numerous so far uncharacterized manifestations in affected mice, hyperammonemia is one of the most theoretically obvious. Due to absent portal venous uptake, several substances are not delivered to liver for processing. In the case of excess ammonia in the CNS, astrocyte specific glutamine synthethase detoxifies ammonia to glutamine (GLN) and increase of it is reliably detectable by proton MR spectroscopy (1H-MRS) *in vivo*. Our results confirm seriously altered brain metabolic profile, and extend seen *in vivo* effects everything from altered bodyweight and brain volumes to defective liver enzymes, different brain FDG-PET readouts and affected behavior. Our material consisted 119 five weeks old male C57Bl/6J mice from which total of 11 mice (9.2%) were diagnosed with portal vein shunt using high GLN levels in 1H-MRS study at 11.7T field strength. Body weight matched control group with normal GLN levels was established and all study mice went through the MR angiography that confirmed the absence of normal portal circulation in all high GLN mice. Brain volumes were longitudinally assessed at 5, 8, 12 and 16 weeks and PS mice had consistently higher brain volumes. PS mice gained more weight from early phase of the study and showed behavioral alterations in the rotarod and open-field. FDG-PET signal was significantly increased in the PS animals suggesting longer bioavailability of FDG due to defective hepatic circulation. Blood clinical chemistry showed defective liver enzymes, AST and ALT at group level, but with overlap in individuals which rejects this test as a diagnostic tool. Similarly as ammonia, bile acids are supposed to be delivered back to liver through portal vein, and we observed up to 50-fold increase in serum/plasma total bile acids in PS mice with not even close overlap between the groups. Our laboratory has performed vast amount (>10000) of 1H-MRS scans in pure C57Bl/6J and transgenic mouse strains derived from it. After meta-analysis of data, 10% seems valid estimate on pure C57Bl/6J that we have obtained. Worst transgenic cohorts with C57Bl/6J background have been shown close to 50% prevalence of PS. Together these results show that pure C57Bl/6J mice and strains derived from it should be used with extreme care, preferably only after screening out the mice with PS. We propose plasma/serum total bile acids as affordable and widely available screening method for portal shunts in mice.

Disclosures: K. Lehtimäki: None. **J. Puoliväli:** None. **T. Huhtala:** None. **T. Heikkinen:** None. **A. Shatillo:** None. **A. Nurmi:** None. **P. Sweeney:** None.

Poster

632. Blood Flow and Functional Imaging

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 632.13/EEE8

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Instituto Israelita de Ensino e Pesquisa Albert Einstein

Title: An fMRI and movement evaluation of Tai Chi and water aerobics training

Authors: *E. H. KOZASA[1], A. PORT[2], D. SANTAELLA[2], S. LACERDA[2], D. SPECIALLI[2], J. BALARDIN[2], P. LOPES[2], R. AFONSO[2], J. RADVANY[2], E. AMARO JR.[2];
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Abstract: Introduction: Aging is related to changes in brain structure and function, which may lead to cognitive declines, as well as physical and behavioral changes. Physical activity may have an important role in cognitive and physical health during the aging process. Objective: To evaluate Tai Chi and water aerobics training effects in an fMRI and gait laboratory examinations. Methods: A total of 16 elders (age> 60) from 2 independent groups, 8 Tai Chi (TC) and 8 Water Aerobics (WA) were matched by gender, years of education and age. Participants filled questionnaires, were examined in the gait laboratory (subjects underwent the anthropometric measurements protocol requested by Vicon® system for carrying out the three dimensional examination of gait) and were scanned in a 3.0T Siemmens MRI equipment during an fMRI Stroop Word-Color Task. Results: The TC group seamed to need a smaller amplitude range of the main joints during gait, performing the walk task in a more centered way, and maybe with smaller energy expenditure than the WA group; the TC group maintained joints closer to a neutral position during the entire step cycle than the WA group. Functional magnetic resonance imaging results showed smaller activation in attention areas in the TC when compared to the WA, confirming the smaller cognitive load during the Stroop Word Color Task in the TC group. Conclusion: Tai Chi may help practitioners to develop a more centered movement related to the body axis, a more neutral use of joints, more gravity-related embodiement and a more effective use of attentional systems compared with Water Aerobics.

Disclosures: E.H. Kozasa: None. **A. Port:** None. **D. Santaella:** None. **S. Lacerda:** None. **D. Specialli:** None. **J. Balardin:** None. **P. Lopes:** None. **R. Afonso:** None. **J. Radvany:** None. **E. Amaro Jr.:** None.

Poster

632. Blood Flow and Functional Imaging

Location: Halls B-H

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Program#/Poster#: 632.14/EEE9

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: MRC Grant 141109

Title: Investigating the role of cortical temperature in neurovascular function and the mechanisms underpinning the anti-epileptic effects of focal cerebral cooling.

Authors: *L. W. BOORMAN[1], S. HARRIS[1], A. KENNERLEY[1], P. REDGRAVE[1], C. MARTIN[1], T. H. SCHWARTZ[2], J. BERWICK[1];
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Abstract: Understanding how changes in cortical temperature affect neurovascular function is relevant to the calibration, development, and interpretation, of existing and novel functional neuroimaging techniques, and can offer insights into neurological disease progression and prevention. Here, we address this issue by employing a novel multi-modal methodology that allows the examination of cortical temperature, tissue oxygenation and blood flow concurrently with laminar electrophysiology and spatial measures of hemoglobin concentration. Multi-modal measures were assessed in the whisker barrel cortex of the urethane-anesthetized rat during sensory stimulation, (16s, 5Hz, 1.2mA), hypercapnia challenge (5 and 10%) and recurrent acute neocortical seizures induced by infusion of 4-aminopyridine (4-AP, 15mM, 1µl). Significant increases ($p < 0.01$) in cortical temperature were observed during sensory stimulation (0.19 ± 0.02^{o}C), 5% hypercapnia (0.89 ± 0.11^{o}C), 10% hypercapnia (1.46 ± 0.19^{o}C), and recurrent seizures (1.78 ± 0.08^{o}C). Cortical temperature increases between conditions were significantly different ($p < 0.05$) with the exception of 10% hypercapnia and the ictal condition ($p = 0.36$). Hypercapnic challenges induced a dose dependent increase in cerebral hemodynamics and decrease in spontaneous neuronal activity, and changes in cerebral metabolic rate. Uncoupling between cerebral blood flow (CBF) and total hemoglobin concentration (Hbt) was seen during the return to baseline at higher levels of hypercapnia and during seizure activity. Complex changes in cortical tissue oxygenation were also observed during seizure initiation and propagation. Interestingly, increases in cortical temperature were more closely coupled to increases in Hbt than CBF, possibly due to inflow of core-temperature blood and increased capillary hyperemia. On-going research involves cortical superfusion of temperature-controlled saline to actively manipulate cortical temperature and examine the role of thermoregulation in neurovascular coupling and the therapeutic benefits of cortical cooling in neocortical epilepsy. Our results have important implications for pre-clinical functional neuroimaging methods in health and disease, and elucidate the role of cortical temperature in the maintenance of normal neurovascular function.

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Poster

632. Blood Flow and Functional Imaging

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Program#/Poster#: 632.15/EEE10

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant Y1AA-3009

Title: Intravenous methylphenidate reduces local functional connectivity density in the Thalamus: An fMRI study

Authors: S. B. DEMIRAL[1], D. TOMASI[1], C. WIERS[1], E. SHOKRI-KOJORI[1], G.-J. WANG[1], *N. D. VOLKOW[2];
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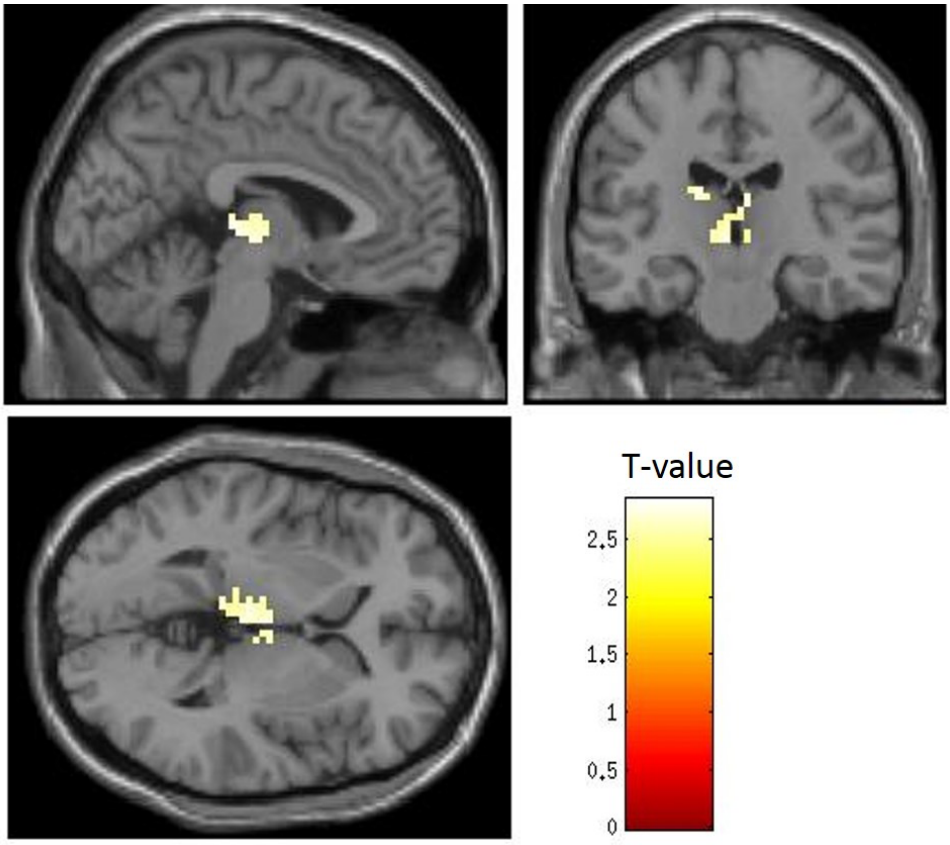
Abstract: Recent functional magnetic resonance imaging (fMRI) studies (Farr et al., 2014; Mueller et al., 2014) showed that the oral administration of methylphenidate (MPH), a dopamine agonist, induced contrastive effects on resting state brain functional connectivity. For instance connectivity within thalamus-dorsal attention networks increased, while connectivity in striato-thalamo-cortical networks decreased. Given the incomplete understanding of the potential effects of MPH on human brain function, and its increasing importance in the treatment of ADHD and similar neurological diseases (Rubia et al., 2009), this study aimed to examine, for the first time, the impact MPH (0.5 mg/kg, iv) on local functional connectivity density (lfcd), a connectivity measure emphasizing the similarities between neighboring voxel activations (Tomasi & Volkow, 2010). In a controlled single-blinded settings, we ran a resting state fMRI study in cannabis abusers (n=13) and in healthy controls (n=16) after iv placebo and on a separate day after iv MPH. Standard fMRI preprocessing routines were applied including motion correction and scrubbing (Power et al., 2014), global signal was taken out, and 6mm smoothing, lfcd threshold was set to 0.6. Our results show that, compared to placebo (PL), MPH decreased lfcd in the Thalamus (total n=29, uncorrected cluster-level p-value 0.002 as found in a t-test using PL-MPH contrast including all subjects). We were not able to find any interaction between PL/MPH administration and cannabis/healthy groups in a 2X2 flexible factorial analysis, using drug administration as within subject factor, and group as a between subject factor. We argue that MPH is potentially minimizing the similarity of the activation patterns between the neighboring voxels in the thalamus thus changing the sub-regional specificity and pace-making routines in cortico-thalamo-cortical loops. Overall, our results indicate that iv MPH has similar effects as oral MPH, and that its influence on lfcd is most pronounced in the thalamus.



Disclosures: **S.B. Demiral:** None. **D. Tomasi:** None. **C. Wiers:** None. **E. Shokri-Kojori:** None. **G. Wang:** None. **N.D. Volkow:** None.

Poster

632. Blood Flow and Functional Imaging

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 632.16/EEE11

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: KAKENHI #26460695

Title: Attenuation of descending and limbic pain modulatory activities during offset analgesia in chronic pain patients: a functional magnetic resonance imaging study

Authors: ***S. ZHANG**, E. IKEDA, T. LI, H. KOBINATA, T. OTA, K. MAKITA, J. KURATA; Anesthesiol., Tokyo Med. and Dent. Univ., Tokyo, Japan

Abstract: Chronification of pain potentially involves pathological modification of the descending pain inhibitory system (DPIS). We examined cerebral substrates of such modification by functional magnetic resonance imaging (fMRI) and a thermal stimulation paradigm causing offset analgesia (OA), an endogenous pain inhibition characterized by a disproportionately large reduction in pain perception after a small decrease in thermal pain stimulus. [Method] We recruited 17 chronic pain patients (CP) and age-gender-matched 17 healthy control subjects (HC). We recorded their psychophysical variables including PainDETECT questionnaire (PD-Q), Short-Form McGill Pain Questionnaire (MPQ), Pain Catastrophizing Scale (PCS) and Beck Depression Inventory (BDI). We gave a mixture of peudorandom thermal pain stimulation paradigm, including 3 blocks of OA and 6 other sham blocks, on the left volar forearm of a subject while obtaining whole-brain fMRI on a 3.0 Tesla scanner. We used a thermal stimulator (Medoc, Israel) with a 3 cm-diameter probe and digital continuous recorder of pain ratings between 0 and 10. The OA block consisted of a series of 5-s 46ºC (T1), 5-s 47ºC (T2), and 20-s 46ºC stimuli (T3), between 32ºC-rest conditions. The sham blocks consisted of three 30-s 46ºC stimuli and three 10-s stimuli with T1+T2 only. Functional images were compared between the groups with fixed-effects general linear model analysis at a threshold of uncorrected $p < 0.00001$ by BrainVoyager QX (BrainInnovation, Netherlands). [Results] During OA, we observed a rapid, disproportionate decrease of pain rating on T3 in both groups. Although the magnitudes of OA were comparable, the pain rating was approximately 30% slower in CP than in HS. During OA, the CP showed deactivation at the bilateral medial prefrontal cortex (mPFC), left posterior cingulate cortex (PCC), right dorsolateral prefrontal cortex (DLPFC), right hippocampus, and right amygdala, while the HC showed stationary or positive activation in those areas. On the other hand, bilateral fusiform gyri showed stationary activation in CP but activated robustly in HC. The blood oxygenation level-dependent amplitudes at the maximal OA response of CP was negatively correlated with MPQ at the PCC, positively correlated with PD-Q at the mPFC, positively correlated with PCS and BDI at the right amygdala ($p<0.05$). [Conclusion] In CP, deactivations at the mPFC, PCC, and DLPFC might represent dysfunction of the DPIS; and those at the subcortical limbic system might be associated with negative emotions. Dysfunction of both the cortical and limbic pain modulatory systems might possibly contribute to the cerebral mechanisms of pain chronification.

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Poster

632. Blood Flow and Functional Imaging

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 632.17/EEE12

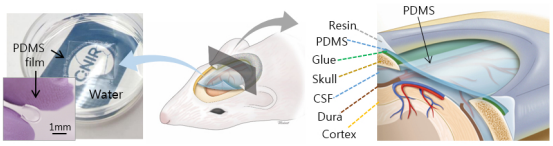
Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: IBS-R015-D1 in Republic of Korea

Title: Soft cranial window development for longitudinal cellular dynamic monitoring

Authors: ***C. HEO**, H. PARK, S.-G. KIM, M. SUH;
Sung Kyun Kwan Univ., Suwon City, Kyeonggi-Do, Korea, Republic of

Abstract: To approach neural tissue to investigate brain function, stable and accessible cranial window is needed for living animal. We developed the new type of cranial window in comparable clarity and stability with conventional glass type window. Big advantage of our soft window is that the window provides multi-site penetration with multiple times by a glass pipette or electrode without causing fluid leakages. The silicon-based soft cover material has sufficient physical properties for penetration by retaining high elastic, flexible, transparent, and biocompatible features. This window can be simply fabricated in laboratory and also easily tailored to any size or shape to cover desired brain area of rat and mouse. We have succeed the longitudinal two-photon imaging for microglial observation before and after chemicals injection into the cortex. In addition, this system allows awake two-photon imaging over 1 hour while the animal was running over treadmill. The soft cranial window showed long term (>3 months) solidity for real-time optical image and electrophysiological recording. Our window technique can provide the new flat form to study various longitudinal and real-time neuroscientific approaches for investigation of dynamic brain function.
Funding: IBS-R015-D1 in Republic of Korea.



Disclosures: **C. Heo:** None. **H. Park:** None. **S. Kim:** None. **M. Suh:** None.

Poster

632. Blood Flow and Functional Imaging

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PENN IOA Pilot Project

program for professors of special appointment (Eastern Scholar) at Shanghai Institutions of Higher Learning

International Program for Ph.D. Candidates, Sun Yat-Sen University

Title: Test-retest reproducibility of cerebral blood flow during a sustained attention task

Authors: *F. N. YANG[1,3,2], S. XU[1,4,2], J. DETRE[1,2], H. RAO[1,4,2];
[1]Dept. of Neurol., [2]Ctr. for Functional Neuroimaging, Univ. of Pennsylvania, Philadelphia, PA; [3]Dept. of Psychology, Sun Yat-sen Univ., Guangzhou, China; [4]Lab. of Applied Brain and Cognitive Sci., Shanghai Intl. Studies Univ., Shanghai, China

Abstract: Introduction: Cerebral blood flow (CBF) measurements have been widely used as biomarkers of neural activity in cognitive and clinical neuroscience studies. Arterial spin labeling (ASL) perfusion MRI can noninvasively quantify CBF thus is increasingly used to access regional brain function in humans. Previous studies have consistently demonstrated high test-retest reliability of quantitative CBF over time. However, the reproducibility of task-induced CBF activation remain unclear. Here we aimed to quantify test-retest reliability of absolute CBF values and relative CBF activation levels induced by a psychomotor vigilance test (PVT) during a well-controlled protocol. **Method**: We analyzed data from 15 healthy control subjects (6 females, mean age = 35±9 years) from a 5-day and 4-night sleep study. Participants slept 8-9 hours every night in the laboratory. Their waking and diet behaviors were continuously monitored throughout the experiment. Each subject were scanned three times on the morning of days 2, 3, and 5. Both voxel-wise and region-of-interest (ROI) analyses were conducted. Intra-class correlation coefficients (ICC) were calculated to quantify test-retest reliability across three scans. **Results**: PVT performance demonstrated very high test-retest reliability (ICC values ranged from 0.870 to 0.936) across three scans. Voxel-wise analysis of the absolute CBF values also showed very high test-retest reliability for both resting and PVT scans, with the ICC values

slightly higher during the PVT (median ICC = 0.932) than at rest (median ICC = 0.908 and 0.907). However, the test-retest reliability of relative CBF changes with task was much lower from both voxel-wise and ROI analyses, with ICC values ranging from 0 to 0.648 for the PVT-induced activation and deactivation clusters. **Conclusion:** Regardless of resting or task scans, absolute CBF showed excellent reproducibility across three scans with ICC values comparable to those of PVT performance. which are consistent with previous studies. However, task induced changes in regional CBF during the PVT showed much lower reproducibility, suggesting that PVT performance may not be maintained by a consistent neural substrate. These findings suggest a preference of using absolute CBF over relative CBF activation as a biomarker for regional brain function in future studies.

Disclosures: **F.N. Yang:** None. **S. Xu:** None. **J. Detre:** None. **H. Rao:** None.

Poster

632. Blood Flow and Functional Imaging

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Support: Research Corporation for Science Advancement Award

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Title: Simulating the light propagation in rodent brain tissues using ray tracing with a 3D microvasculature model

Authors: *P. TIAN[1,2], S. IFEANYI[1], T. J. SAUER[1], W. COTTON[1], A. DEVOR[2,3,4], Q. FANG[4], H. UHLIROVA[3], P. A. SAISAN[2], A. M. DALE[3], D. A. BOAS[4], S. SAKADZIC[4];
[1]John Carroll Univ., University Heights, OH; [2]Neurosciences, [3]Radiology, UCSD, La Jolla, CA; [4]Martinos Ctr. for Biomed. Imaging, MGH, Harvard Med. Sch., Charlestown, MA

Abstract: The advent of two-photon fluorescence microscopy has opened unprecedented opportunities in revealing neuronal firing, hemodynamic changes, and metabolic activity on microscopic level *in vivo*. However, data interpretation of two-photon fluorescence microscopy on dyes with small signal change such as *β-nicotinamide adenine dinucleotide* (NADH), a key metabolic marker, faces enormous challenge because the measured signal change is often highly distorted by hemodynamic changes. Prior work [Baraghis et al, Journal of Biomedical Optics 16, 106003 (2011) and Sauer et al, SFN 2014] modeled two-photon NADH fluorescence with precise maps of cortical microvasculature and corrected for the measured NADH signal change by using the fluorescence change of Sulforhodamine 101 (SR101), a functionally inert dye topically applied to the cortex. However, we only simulated for one animal model (a rat) and one dye, NADH. Here, we extend the prior work to systematically calculate the point to point correction factor using real 3D microvasculatures in two animal models (rats and mice) and two different dyes (NADH and Oregon Green 488 BAPTA-1, OGB). We have found that for both animal models and dyes: 1) the correction factors vary significantly with the appearance of large vessels near the pial surface while they tend to be more homogeneous at deeper depths (Figure: 1st three columns show the correction factor at 40, 60, 80, 100, 120, 140, 200, 300 and 400 microns. 4th column shows the corresponding vasculatures). 2) a single-value correction factor may be used to effectively correct the hemodynamic distortion in layer II or deeper depths. The residual error after the correction is comparable to the experimental error. Our study may help quantify cellular NADH signal change and is also applicable to two-photon fluorescent measurements where changes of tissue optical properties affect measured signals, thus allowing more accurate interpretation of functional imaging studies.

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Poster

632. Blood Flow and Functional Imaging

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Japan Society for the Promotion of Science (JSPS) Graint-in-Aid for Exploratory Research (16K12970)

Title: Assessment of brain activities using short-term gray matter changes

Authors: *Y. KABURAKI[1], T. ONO[2], E. YAMADA[2], H. EBATA[2], D. NISHIDA[2], S. KASUGA[1], J. USHIBA[3];
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Abstract: Voxel-based morphometry (VBM) analysis of magnetic resonance images (MRI) has revealed structural changes in the brain gray matter following visuomotor task (Draganski et al., 2004). Recently, we found such gray matter changes occur even with relatively short-term (i.e., hours) visuomotor task (Kodama et al. in prep). Possible neurobiological factors behind such short-term structural changes may be changes in astrocytes or vessels (Berg et al., 2012), which is due to metabolic processes accompanying with neural activities in the corresponding area. It is therefore suggested a possible use of VBM as a functional measurement of a whole brain activity. In order to test its feasibility, the present study evaluated correlations between gray matter volume increase and blood oxygenation level dependent signals during saccadic eye movement task, and tested the similarity of these images. Twelve healthy volunteers participated in the study. Participants performed a total of 288 trials (six blocks) of saccade task in the MR machine. At the start of each block, the participant fixated a dot displayed at the center of a computer monitor in front of their face. After the fixation dot disappeared, a saccade target appeared rightward or leftward of the center. Dependent on the color of the fixation dot, participants were instructed to look at the opposite direction or the same direction of the target. We acquired fMRI BOLD signals during the task, and T1-weighted structural MR images for VBM immediately before and after the task. In VBM analysis, significant gray matter increases after an hour of saccade training were detected in the visual cortex, the supplementary eye field ($p<.001$, unc), the intraparietal sulcus and the basal ganglia, which is previously known to be involved in saccade tasks (Deseilligny et al., 2004). In fMRI analysis, task-related brain activities were detected in the similar brain regions to VBM analysis, suggesting that gray matter changes reflect neuronal activities in these regions. In contrast, gray matter increase was detected in the right thalamus, whereas fMRI analysis did not show significant BOLD changes. This difference may result from characteristic of the temporal pattern of neuronal activity in this region, which may be hardly detected by fMRI. Our findings open up new possibilities for functional brain imaging during motor activities, because VBM does not require movement constraint during the motor tasks like fMRI, nor invasive procedures like positron emission tomography. Future investigations are expected to evaluate quantitative relationship between motor learning or the motor activity level and gray matter volume increases.

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Poster

633. Neuroimaging of Reward Mechanisms

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 633.01/FFF2

Topic: G.02. Motivation

Title: Hedonic and utilitarian values in the human brain

Authors: *K. MOTOKI, R. KAWASHIMA, M. SUGIURA;
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Abstract: Consumer choices are driven by hedonic or utilitarian values. People buy hedonic goods for obtaining emotional experiences such as joy, or excitement, while they pursue utilitarian goods by pursuing functional or instrumental needs. Consumer researchers suggest that these different values map onto independent components of value dimensions. However, there is a hypothesis that common specific brain regions encode different types of value of goods. Especially, it has been suggested that ventromedial prefrontal cortex (vmPFC), and bilateral ventral striatum (VS) encode a wide range of economic values, when people explicitly engage in purchasing decisions (paying money for the goods). Although previous studies examined different category of goods, they have not differentiated hedonic and utilitarian value dimensions. Therefore, it remains uninvestigated which brain regions encode explicit value representations dependent or independent of hedonic/utilitarian value dimensions. In addition to that, the same brain regions (vmPFC and VS) are considered to capture automatic economic values when explicit economic evaluations are not required. However, it remains elusive which brain regions encode automatic value representations dependent or independent of hedonic/utilitarian value dimensions. We addressed these questions by scanning participants with functional magnetic resonance imaging. We investigated the brain activations during the processing of economic valuations in both hedonic and utilitarian goods (comic books for hedonic goods and how-to-book for utilitarian goods) at explicit and automatic stages. Participants performed two decision-making task across hedonic and utilitarian goods in which they made purchasing decisions (for which values are directly explicit) or perceptual decisions (for which values are unrelated to the purchasing decisions and therefore value representations are automatic). We found vmPFC and VS to encode explicit value representations independent of hedonic/utilitarian goods, but no unique brain regions to contain explicit value representations for either hedonic or utilitarian goods. We did not find brain regions to contain automatic value representations independent hedonic/utilitarian goods. While we did not find brain regions to contain automatic value representations uniquely for utilitarian goods, vmPFC encoded automatic value representations selectively for hedonic goods. Such a commensurable nature of explicit values and an automatic value representation selectively for hedonic dimension might provide insight into consumer decision theory based on neural evidences.

Disclosures: **K. Motoki:** None. **R. Kawashima:** None. **M. Sugiura:** None.

Poster

633. Neuroimaging of Reward Mechanisms

Location: Halls B-H

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Program#/Poster#: 633.02/FFF3

Topic: H.02. Human Cognition and Behavior

Title: Biased performance monitoring of physical effort in chronic fatigue

Authors: ***M. E. VAN DER SCHAAF**[1,2], I. TONI[3], F. P. DE LANGE[3], K. ROELOFS[3], J. W. M. VAN DER MEER[4], H. KNOOP[5];
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Abstract: Chronic fatigue syndrome (CFS) is characterized by profound and disabling fatigue leading to reductions in physical performances that are not explained by lack of fitness or muscular fatigue. Clinical work has suggested that these behavioural changes arise from dysfunctional beliefs about the ability to perform physically demanding tasks.
Here, we explore how such beliefs bias feedback-driven behavioural adjustments during an effortful physical exertion task. 85 CFS patients and 29 controls exerted grip force at 30, 50 and 70% of their maximal voluntary contraction (MVC) and received directional feedback on their performance (too much, too little, or correct). Beliefs were indexed with a clinical questionnaire assessing expected levels of fatigue evoked by various daily physical tasks (PARS). Neural correlates of biases in feedback-driven effort adjustment were assessed with fMRI.
CFS patients showed a stronger force-related bias in feedback-driven effort adjustments than controls (force*group: $F_{2,111} = 7.04$, $p<.01$), while MVC (change) was matched across groups ($p>.1$). This bias was present only during trials requiring 70% of MVC. CFS patients showed smaller increases in force production after feedback indicating that too little force was produced, and larger reductions after feedback indicating that too much force was produced ($T_{111} = -2.65$, $p<.01$). Neurally, CFS-related bias was associated with weaker feedback-related signals in the dorsolateral prefrontal cortex (DLPFC, bilaterally, $p<.015$ family-wise error-corrected). Within the CFS group, larger force-related biases in feedback-driven effort adjustments were associated with lower DLPFC activity ($r = .25$, $p<.02$). CFS patients also report higher levels of fatigue expectations than HC ($T_{111}= 14.8$, $p<.001$), and higher fatigue expectations were associated with

larger behavioural biases in CFS patients ($r = -.31$, $p<.01$).
These findings show that chronic fatigue is associated with biases in feedback-driven effort-related behaviour. The DLPFC may play a critical role in biasing error-related adjustments to fit with prior beliefs about the ability to perform the task. These findings open the possibility of using objective behavioural and neural markers to index changes in CFS during therapeutic interventions that aim to change dysfunctional beliefs like cognitive behavioural therapy.

Disclosures: M.E. Van Der Schaaf: None. **I. Toni:** None. **F.P. De Lange:** None. **K. Roelofs:** None. **J.W.M. van der meer:** None. **H. Knoop:** None.

Poster

633. Neuroimaging of Reward Mechanisms

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 633.03/FFF4

Topic: G.02. Motivation

Support: Masud Husain Wellcome Trust Principal Fellowship

Title: Effort but not reward sensitivity is altered by sickness behaviour in humans

Authors: *A. DRAPER[1], R. M. KOCH[2], J. W. M. VAN DER MEER[3], P. PICKKERS[2], M. HUSAIN[1], M. E. VAN DER SCHAAF[4];
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Abstract: Sickness in humans, associated for example with the systematic inflammatory responses to infection, is characterized by low mood and fatigue. At the behavioural level, there may be changes in motivation involving the reorganization of priorities but it is unclear which specific processes underlying motivation are altered. In rodents, recent work suggests that experimentally induced inflammation reduces high-effort choices, crucially without affecting reward preference[1].
Here, we examined the effect of systemic inflammation induced by experimental endotoxemia in humans. We tested whether bacterial endotoxin e. Coli lipopolysaccharide (LPS) affected effort or reward sensitivity using an effort-stake choice paradigm[2]. Participants first familiarised themselves with 5 different effort levels using hand-held dynamometers which they squeezed. On a trial-by-trial basis, they then made a series of decisions on whether the stake offered (5 levels) was “worth the effort” (5 levels). Of 100 offers, 26 were selected to be performed afterwards. 29 healthy young males were administered either LPS (2ng/kg; n=14) or placebo

(0.9% saline; n=15). The effort-stake task was assessed prior to LPS infusion, 2 and 5 hours post-infusion. Relationships between the acute cytokine responses (plasma levels of TNFα, IL6, IL8, IL10, IL1-ra) and changes in motivation and self-reported mood (depression and fatigue scales) were also assessed.
Experimental endotoxemia resulted in a 1.3°c increase in temperature, and marked increases in all cytokines measured. After 2 hrs, systemic inflammation increased effort ($F=3.2$, $p<0.05$) but not reward ($F=0.5$, $p>0.7$) sensitivity relative to baseline and placebo. Importantly, LPS reduced willingness to accept high effort options, irrespective of reward. These group differences were absent by 5 hrs suggesting partial recovery. The change in effort sensitivity was linked to the extent of the pro-inflammatory response. Both IL6 and IL8 levels predicted the influence of effort on decisions made at 2 hrs ($R^2=0.6$). Self-reported fatigue and depression were also increased then and associated with TNFα levels ($R^2=0.4$).
These results show that in humans inflammation affects effort but not reward sensitivity and that these changes are predicted by the acute pro-inflammatory cytokine response during experimental endotoxemia. The findings provide mechanistic insight into understanding motivational changes during sickness.
1. Vichaya et al (2014), Neuropsychopharmacology, 39: 2884-2890.
2. Bonnelle et al (2016), Cerebral Cortex, 26 (2): 807-819.

Disclosures: **A. Draper:** None. **R.M. Koch:** None. **J.W.M. van der Meer:** None. **P. Pickkers:** None. **M. Husain:** None. **M.E. van der Schaaf:** None.

Poster

633. Neuroimaging of Reward Mechanisms

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 633.04/FFF5

Topic: G.02. Motivation

Title: Dissociating effort and reward signals in the striatum

Authors: *A. R. ARULPRAGASAM[1], D. J. COLE[2], N. IRFAN[2], M. T. TREADWAY[2];
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Abstract: Dopamine (DA) functioning within the striatum is believed to play a critical role in effort-based decision-making and effort-discounting. However, despite considerable research demonstrating DA's involvement in motivated behavior, the specifics of this involvement are not fully understood. While research implicates midbrain DA neurons in valuation-related aspects of decision-making and in modulating the performance of reward-seeking behaviors, growing evidence suggests that DA is also required to mobilize effort expenditure in pursuit of rewards.

To better understand how the striatum responds to effort and reward during effort-based choice, 30 healthy participants completed a sequential effort-based decision-making task while undergoing functional magnetic resonance imaging (fMRI). In this task, participants completed a series of trials in which they chose between performing no effort for a fixed small reward or 20%, 50%, 80% or 100% effort for a larger, variable reward. For each trial, information about the high-reward/high-effort option was presented sequentially; one piece of information (effort level or reward magnitude) was presented first followed by a jittered ISI and then the other piece of information. Order was counterbalanced across all levels of effort and reward. The advantage of this design is that it allows for the isolation of neural responses to effort or reward alone, and their subsequent integration. Using this task, we were interested to see how the striatum responds to the addition of high effort or low reward information following high reward or low effort presentation.

If striatal signals primarily encode discounted utility function, we might expect decreased striatal activity following the addition of high effort or low reward. Consistent with this, we observed increased nucleus accumbens activity when low effort information was presented first when compared to the addition of low reward ($p_{FWE} = 0.038$, SVC). In contrast, if the striatum also encodes the value of overcoming effortful response costs, we might expect greater striatal activity when high effort information is added. This was also observed in anterior caudate during trials in which high reward values were followed by the presentation of high effort information ($p_{FDR}<0.05$, SVC).

Taken together, these results suggest the coexistence of discounting and effort mobilization signals in the striatum. Importantly, these signals were anatomically segregated, possibly implicating differential involvement of DAergic signaling. These data have the potential to increase understanding of the neurobiological mechanisms underlying motivated decision-making.

Disclosures: **A.R. Arulpragasam:** None. **D.J. Cole:** None. **N. Irfan:** None. **M.T. Treadway:** None.

Poster

633. Neuroimaging of Reward Mechanisms

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Program#/Poster#: 633.05/FFF6

Topic: G.02. Motivation

Support: BBSRC fellowship (BB/M013596/1)

Wellcome Trust Principal Research Fellowship (098282/Z/12/Z)

Title: Neural mechanisms of subjective reward devaluation by cognitive and physical effort

Authors: ***M. A. APPS**[1], T. T.-J. CHONG[2], K. GIEHL[3], L. GRIMA[1], M. HUSAIN[1];
[1]Univ. of Oxford, Oxford, United Kingdom; [2]Macqaurie Univ., Sydney, Australia; [3]Univ. of Cologne, Cologne, Germany

Abstract: Animals and humans have to constantly make decisions about whether it is worth exerting effort to obtain rewards. Such subjective evaluations (SV) of the cost and benefit of exerting effort to obtain a reward are central to motivated behavior. Yet, effort must be exerted in different domains of behaviour. In the modern era, countless tasks we perform are highly cognitively demanding and require minimal physical exertion, but the converse is also true for many of other behaviours. However, very little is known about the computational or neural basis of how different effort costs are subjectively weighed against rewards. Is there a common, domain-general system of brain areas that evaluates all costs and benefits? We used computational modelling and functional magnetic resonance imaging (fMRI) to examine the mechanisms underlying SV processing in both the cognitive and physical domains.
Training - Participants trained on two tasks which parametrically varied in cognitive or physical effort and were rewarded if performance met a pre-specified criteria. For the cognitive task, subjects were required to make peripheral switches of attention to detect targets during a 14s trial (Apps et al., 2015, *Sci. Reports*). For the physical task subjects were required to sustain a force grip at one of six different percentages of their maximum voluntary contraction for 14s (Chong et al., 2015, *Cortex*).
Scanning - During fMRI, participants indicated their preferences between a fixed low-effort/low-reward option and a variable higher-effort/higher-reward offer separately for each effort domain. 10 choices for each task were randomly selected and executed after scanning.
Results and Conclusions - Using model comparison approaches we were able to show that individuals' motivation is significantly correlated between domains, but rewards were valued differently within each domain. The devaluation of rewards was hyperbolic for cognitive effort but parabolic for physical effort. We examined activity covarying trial by trial with SV according to the model. Strikingly, the SV of both types of effort was processed by a largely common network of areas including dorsomedial and dorsolateral prefrontal cortex, intraparietal sulcus and anterior insula. However, we also identified a unique, domain-specific role of the amygdala for processing the SV of rewards only when associated with cognitive effort. These results are the first to reveal the neurocomputational mechanisms underlying subjective cost-benefit valuation across different domains of effort, and provide insight into the multidimensional nature of motivation.

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Poster

633. Neuroimaging of Reward Mechanisms

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Topic: G.02. Motivation

Support: KAKENHI No. 15H05876

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Strategic Japanese - German Cooperative Programme of AMED

Title: Forgetting in reinforcement learning reconciles the two roles of dopamine:reward prediction error and motivational drive

Authors: *A. KATO[1], K. MORITA[2];
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Abstract: It has been considered that dopamine (DA) has two distinct roles, (1) representing reward-prediction-error (RPE) and (2) providing motivational drive. Normative theories have been proposed for both the role as RPE and the role as motivational drive in the framework of reinforcement learning (RL). On the other hand, from a bottom-up, mechanistic point of view, underlying cellular/circuit mechanisms for the motivational role remain more elusive than those for the role as RPE. Here, we explored a unified mechanistic account for the DA's two roles in the framework of RL, with an assumption that DA, not only phasic DA but also recently observed sustained DA that has been suggested to represent motivational drive, consistently represents RPE. Specifically, in the framework of RL, we examined the effects of the decay/forgetting of leaned values that was shown in our previous study to cause sustained RPE, which could explain the observed sustained DA. To examine behavior that can reflect the level of motivation, we modeled self-paced approach towards a goal as a series of 'Go' or 'No-Go' (or 'Stay') selections, and defined 'motivation' as the time (number of time steps) required for goal-reaching. Through simulations, we found that the value-decay can enhance 'motivation', i.e., can facilitate fast goal-reaching. Mathematical analyses revealed that there are two underlying potential mechanisms: (1) a gradient of 'Go' values towards a goal generated by decay-induced sustained RPE, and (2) value-contrast between 'Go' and 'No-Go' generated because chosen values are continually updated whereas unchosen values simply decay. Our model can potentially explain several key experimental findings that suggest the DA's role as motivational drive, specifically, (i) slowdown of behavior by post-training blockade of DA signaling, (ii) severe impairment of costly seeking distant rewards but not of seeking/liking immediate rewards by DA blockade, and (iii) relationships between reward amounts, motivation levels, and DA

levels. Since DA was assumed to consistently represent RPE in our model, these results suggest that the DA's two roles, RPE and motivational drive, can be coherently understood in the framework of RL, provided there exists decay/forgetting of learned values that can be implemented as synaptic decay.

Disclosures: **A. Kato:** None. **K. Morita:** None.

Poster

633. Neuroimaging of Reward Mechanisms

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

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Topic: G.02. Motivation

Support: Lieber Institute for Brain Development

Title: A single nucleotide polymorphism in NKCC1 is associated with motivation-related activity in the human midbrain

Authors: ***R. W. LEFCO**, K. L. BIGOS, Q. CHEN, D. R. WEINBERGER, C. F. ZINK; Clin. Sci., Lieber Inst. For Brain Develop., Baltimore, MD

Abstract: Motivation deficits are a prominent negative symptom of schizophrenia, and it is therefore of clinical relevance to determine the genetic influences underlying motivation-related neural activity. Functional magnetic resonance imaging (fMRI) studies have revealed that activation of the ventral striatum (vStr) and the ventral tegmental area/substantia nigra (VTA/SN) region of the midbrain are associated with motivational processes in humans. Recently, we used random forest multiple regression to determine the influence of multi-genetic variation within distinct neurotransmitter gene-sets on motivation-evoked fMRI signals in the VTA/SN midbrain and vStr. It was determined that multi-genetic effects/epistasis in the GABA gene-set account for 3.66% of variance in motivation-related midbrain signal ($p_{empirical} = 0.00$). Within these genes, a functional single nucleotide polymorphism (SNP) in the GABAa receptor was identified as most influential, thus implicating GABAa receptor signaling as important in the neural basis of motivational processes. NKCC1 is an ion channel that can mediate GABAa receptor activity indirectly by modulating intracellular Cl- concentration, and the minor allele of a functional NKCC1 SNP, rs3087889, has been associated with increased schizophrenia risk. Therefore, in the current study, we investigated the influence of the rs3087889 NKCC1 SNP on motivation-related midbrain activity using a variation of the monetary incentive delay (MID) task in fMRI. We isolated motivation-related (high motivation cues > low motivation cues) midbrain activity in healthy subjects (n = 86) and performed a multiple regression analysis in

which this activity was regressed on rs3087889 genotype. We found that the risk-associated allele (*A*) of rs3087889 significantly predicted reduced VTA/SN midbrain motivation-related activity [peak = -6, -22, -22, t(84) = 2.54; $p < 0.01$]. These results implicate genetic variation in the NKCC1 gene as a determinant of motivation-related midbrain activity, and, because the minor allele of rs3087889 is associated with schizophrenia risk, may be relevant to the genetic basis of motivation deficits in schizophrenia.

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Poster

633. Neuroimaging of Reward Mechanisms

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Program#/Poster#: 633.08/FFF9

Topic: G.02. Motivation

Support: Stanford Center for Cognitive and Neurobiology Imaging Grant to YCL

Title: Seeing what we want to see: Motivation influences visual perception via changes in neural gain

Authors: *Y. LEONG, B. L. HUGHES, J. ZAKI;
Dept. of Psychology, Stanford Univ., Stanford, CA

Abstract: People tend to think of visual perception as a veridical representation of the physical world, but what we want can influence what we see. Previous work demonstrates that the perception of ambiguous or bistable visual stimuli is biased in favor of percepts associated with positive outcomes. In this study, we explored the neural mechanisms underlying motivational influences on visual perception. We investigated if motivation enhances preparatory neural activity selective to desired perceptual features in anticipation of a stimulus, or if it increases the neural sensitivity to those features during stimulus presentation.
Human participants performed a visual categorization task with images comprising a mixture of a face and a scene in different proportions. For each image, participants were rewarded for correctly indicating whether the image predominantly displayed a face or a scene. Prior to seeing each image, a teammate or an opponent placed a bet about whether the upcoming image would predominantly display a face or a scene. For teammate trials, participants won a bonus if teammates bet correctly, but lost money if the teammates bet incorrectly. For opponent trials, participants lost money if opponents bet correctly, but won a bonus if opponents bet incorrectly. As such, participants were motivated to see the type of image consistent with the teammate's

bets, and to see the type of image inconsistent with the opponent's bets. Crucially, the reward maximizing strategy is to ignore the bets and perform the classification as accurately as possible. Nevertheless, we found that the bets shifted participants' sensitivity to the motivation-consistent category - for the same face to scene ratio, participants were more likely to classify an image as belonging to a category if they were motivated to see that category.
We then applied multi-voxel pattern analysis methods to participants' BOLD response to quantify the level of face-selective and scene-selective activity in the ventral visual stream, both when participants saw the bet (pre-stimulus period) and when participants saw the image (stimulus presentation). Category-selective activity during the pre-stimulus period was not higher when participants were motivated to see the category, indicating that motivation did not enhance preparatory category-selective activity. Instead, motivation increased the neural gain to the motivation-consistent category during stimulus presentation. These results suggest that motivation can influence perception, and might do so via gain control mechanisms that increase neural sensitivity to motivation-consistent perceptual features.

Disclosures: Y. Leong: None. **B.L. Hughes:** None. **J. Zaki:** None.

Poster

633. Neuroimaging of Reward Mechanisms

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Program#/Poster#: 633.09/FFF10

Topic: G.02. Motivation

Support: NARSAD Young Investigator Award

DA036996

DA015096

Title: Exploring midbrain circuit dynamics of conditioned motivation

Authors: *B. T. SAUNDERS[1], P. JANAK[1,2];
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Abstract: Dysfunctional conditioned motivational states contribute to a variety of affective disorders, such as addiction, overeating, depression, and compulsive gambling, which collectively inflict a great toll on public health. Despite this, we lack a comprehensive understanding of how the brain creates motivation that can promote psychopathology, a major obstacle to establishing effective treatments. Addiction in particular is characterized by

exaggerated, compulsive drug seeking, often spurred by drug-associated cues, and a persistent threat of relapse. Dopamine neurons originating from midbrain regions like the ventral tegmental area (VTA) and substantia nigra (SN) have received attention for their role in reward-related processes, including addiction, but it remains unclear how heterogeneity within these systems maps onto behavioral function to facilitate conditioned motivation in adaptive reward-seeking states, or maladaptive states like in addiction. In a series of studies, we found that optogenetic activation of VTA DA neurons in TH-cre rats, and specifically those projecting to the nucleus accumbens core, but not shell, is sufficient to instantiate previously neutral Pavlovian cues (e.g. lights and tones) with incentive motivational value, making the cues attractive and reinforcing in their own right. By comparison, cues paired with optogenetic stimulation of SN neurons, including those specifically those projecting to the most dorsal part of the striatum, invigorated movement, but the cues themselves do not become attractive or reinforce actions. In contrast to these divergent conditioned responses, optogenetic stimulation of VTA or SN DA neurons, and also their respective projections to core, shell, and dorsal striatum, similarly reinforces self-stimulation behavior. These results suggest that DA neurons promote distinct conditioned motivational processes as a function of both anatomical location and projection target, but primary reinforcement is consistently mediated by DA neurons throughout the midbrain. In ongoing experiments, we are expanding on these results to understand these systems in the context of addiction. Using in vivo deep brain calcium imaging, we are visualizing the activity of DA and GABA neurons across the VTA and SN, and their projections to striatum, prefrontal cortex, and thalamus, to determine how different midbrain circuits encode cocaine reward and cocaine-seeking behaviors.

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Poster

633. Neuroimaging of Reward Mechanisms

Location: Halls B-H

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Program#/Poster#: 633.10/FFF11

Topic: G.02. Motivation

Support: R21 HD074850

Title: Context-dependent development of mesolimbic network connectivity throughout adolescence.

Authors: *V. P. MURTY[1], D. MONTEZ[2], W. FORAN[2], B. LUNA[2];
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Abstract: The mesolimbic dopamine system continues to mature throughout adolescence into early adulthood. Neuroimaging has characterized increases in ventral striatal activation across adolescence during reward-motivated behaviors; however, relatively less work has investigated interactions between the ventral striatum and the ventral tegmental area (VTA), the source of mesolimbic dopamine neurons. The current study characterized VTA connectivity with its mesolimbic targets across adolescent neurodevelopment. To determine the developmental trajectory of this system as a function of individuals' goal states, we assessed connectivity in both neutral and rewarding contexts. FMRI data was collected in 170 individuals ranging in age between 10-30 years old. Participants completed both a resting-state task (neutral context) and a reward-motivated anti-saccade task (rewarding context). To compare results across tasks, we characterized 'background connectivity of the reward-motivated anti-saccade task, which reflects intrinsic connectivity between regions after removing task-related activity. Results indicate a significant decrease in VTA-ventral striatal coupling in the rewarding context as individual's approached adulthood ($p < 0.001$). Conversely, there were no differences in VTA-ventral striatal coupling as a function of age in the neutral context (i.e., resting state scan, $p = 0.93$). These findings support a model by which connectivity of the VTA with its mesolimbic targets is relatively stable across adolescence, however, the ability to engage this circuit in motivational-relevant contexts continuous to mature into early adulthood. Future analysis will incorporate VTA network connectivity with other mesolimbic targets including ventromedial prefrontal cortex, hippocampus, and dorsal striatum.

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Poster

633. Neuroimaging of Reward Mechanisms

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Topic: G.02. Motivation

Support: Research Foundation Flanders FWO11_PDO_016

European Research Council ERC-2014-StG-636116

Title: Neural dynamics of reward effects on conflict processing: insights from simultaneous EEG and fMRI

Authors: ***H. R. PARK**[1,2], C. N. BOEHLER[1,2], P. VAN MIERLO[1,3], R. M. KREBS[1,2]; [2]Dept. of Exptl. Psychology, [3]Dept. of Electronics and Information Systems, [1]Ghent Univ., Ghent, Belgium

Abstract: Previous research indicates that motivational states are modulated by reward prospects. A large number of behavioural studies have found a robust facilitation effect of reward, which has been observed across many aspects of cognitive control, including conflict processing. Recent research has focused on this relationship between control and motivation, and how these constructs contribute to the selection of specific actions. Neuroimaging findings indicate several cortical regions that are associated with performance in both reward and conflict tasks, further adding to the evidence of a motivation-control link. Despite this, only a few studies have examined this relationship using multimodal methods, which may provide a bridge between the temporal dynamics and the neural correlates of reward and control interactions. Here, we utilised simultaneous EEG-fMRI recording to investigate the influence of reward availability in a picture-word interference (conflict) task in healthy individuals. Event-related potential (ERP) analyses revealed P3 and N450-like components (related to reward- and conflict-processing, respectively). Interestingly, incongruent trials elicited a more positive N450 amplitude compared to the control (congruent and neutral) trials, which was mainly driven by the rewarded incongruent trials, indicating a modulation of conflict by reward. fMRI analyses also identified a similar interaction in the insula and the anterior cingulate cortex (ACC), which showed smaller activation differences between the rewarded incongruent and control trials, compared to non-rewarded trials. Additionally, an interaction was also observed in the midbrain; however, here the difference was larger for the rewarded trials, potentially reflecting increased effort to resolve the conflict. Finally, intermodal correlations were performed between the N450 and ACC, which is suggested to be one of the key mediating regions involved in reward-based decision making. As hypothesised, there was a positive correlation between the ERP and BOLD signal differences for the non-rewarded incongruent minus control condition, where the incongruent trials elicited stronger neural responses in both EEG and fMRI compared to control. However, in the rewarded trials, only the N450 showed a large difference, indicating that ACC activity is strongly driven by reward that may override any conflict-related activations when averaged over time. This suggests that conflict processing within the context of reward is a transient process that may only be detected using temporally sensitive methods, highlighting the importance of multimodal recordings.

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Poster

633. Neuroimaging of Reward Mechanisms

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Topic: G.02. Motivation

Support: NWO VENI M.Luijten 451-15-029

NWO VENI G. Sescousse 451-14-006

NWO VENI A.F. Schellekens

Title: Is reward processing up- or down-regulated in addiction? the answer provided by image-based meta-analysis of fmri studies

Authors: *M. LUIJTEN[1], A. F. SCHELLEKENS[3,4], S. KÜHN[5,6], M. W. J. MACHIELSEN[7], G. SESCOUSSE[2];
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Abstract: **Importance:** Disrupted brain reward processing, mainly driven by ventral striatal (VS) dysfunction, is considered a key process in addictive behaviours. However, conflicting results have been reported on the exact nature and direction of reward processing disruptions in addiction using functional Magnetic Resonance Imaging (fMRI). In the context of addiction, both hypo and hyper activation have been reported during both anticipation and outcome notification of rewards.
Objective: To identify brain reward processing disruptions during anticipation and outcome notification of monetary rewards in addicted individuals by image-based meta-analysis of fMRI studies. **Data Sources:** Pubmed until January 2015.
Study Selection: Inclusion criteria: 1) reward task involving monetary reward anticipation and/or outcome; 2) addictive behavior including substance addiction or gambling disorder; 3) healthy control group. Exclusion criteria: 1) Age < 18; 2) recreational substance use or gambling; 3) at risk group; 4) patient overlap between studies using the same task.
Data Extraction and Synthesis: Study procedures were conducted in line with the MOOSE guidelines. Using Seed-based *d* Mapping software, meta-analyses were performed with group *T*-maps from individual studies as input (83% response rate from authors for sending group *T*-maps).
Main Outcome and Measures: Estimation of cross-study consistency of between-group differences in reward-related brain activation patterns for reward anticipation and outcome.
Results: 25 studies were included in the meta-analyses representing 643 individuals showing addictive behaviors and 609 healthy controls. Data was analysed using *Seed-based d Mapping* software using non-parametric statistics and with a statistical threshold that offers a protection against false positives that is similar to a corrected $p<0.05$. The main findings include *decreased* VS activation across individuals with different types of addictive behaviour (including substance addiction and gambling) compared with healthy controls during *reward anticipation.* During *reward outcome,* substance addicted individuals showed *increased* activation in the VS compared with healthy controls, whereas individuals with gambling disorder showed *decreased*

activation in the dorsal striatum (DS) compared with healthy controls.
Conclusions and Relevance: These findings cannot be directly reconciled with any of the currently dominant addiction theories, including the Reward Deficiency Syndrome, Incentive Salience Theory or Impulsivity Theory. As such, we believe they are helpful in refining current hypotheses about reward dysfunction in addition.

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Poster

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Support: Stanford Neuroscience Institute NeuroChoice Initiative

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FINRA Investor Education Foundation

Title: Structural and functional neural characterization of the Monetary Incentive Delay Inhibition (MIDI) task

Authors: *J. K. LEONG[1], K. HENNIGAN[1], G. R. SAMANEZ-LARKIN[2], B. KNUTSON[1]; [1]Stanford Univ., Stanford, CA; [2]Yale Univ., New Haven, CT

Abstract: Over a decade of human and monkey studies have shown that the structural integrity of the right ventrolateral prefrontal cortex (vlPFC) is critical for motor inhibition. Most of these studies, however, have not examined inhibition under incentivized conditions. In this study, we imaged structural connections from vlPFC, and functional brain activity associated with motor inhibition to earn monetary incentives (n=23, mean age=37, right-handed). We found that while high incentives enhanced faster motor responses to obtain incentives (paired $t(22)=4.22$, $p<0.001$), they also increased the difficulty of inhibiting motor responses (paired $t(22)=-2.88$, $p<0.01$). We then identified a white-matter tract connecting the vlPFC to the anterior insula (AIns), and also replicated characterization of a tract connecting the AIns to the nucleus accumbens (NAcc). Individual differences in the coherence of both of these tracts, in addition to functional brain activity, were independently associated with individual differences in successful inhibition for high incentives, even after controlling for age ($F(5,17)=35.06$, $p<0.0001$; AIns-NAcc: $B=0.60$, $t=7.73$, $p<0.0001$; vlPFC-AIns: $B=-0.61$, $t=-7.43$, $p<0.0001$; AIns activity:

B=0.23, t=2.52, p<0.05; vlPFC activity: B=0.29, t=3.20, p<0.01; age: B=-0.07, t=-0.9, p=0.40; all coefficients are standardized). However, it was unclear whether functional brain activity during anticipation of high incentives was blunted due to uncertainty in mixed task demands to both motorically respond as well as inhibit responses. To test this possibility, we compared brain activity in the incentivized inhibition (MIDI) task to a monetary incentive delay (MID) task that required only motor responses without inhibition. Contrasts revealed that subjects showed higher NAcc activity during anticipation of equivalently high gains in the MIDI vs the MID task (VOI analysis: paired t(22)=1.96, p=0.06). Together, these findings validate a new incentivized inhibition task, indicate that knowledge about brain structure can guide functional and behavioral models, and clarify multiple brain systems that subserve incentivized inhibition.

Disclosures: J.K. Leong: None. **K. Hennigan:** None. **G.R. Samanez-Larkin:** None. **B. Knutson:** None.

Poster

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Support: European FET flagship project Human Brain Project (604102)

The Israel Center Of Research Excellence in Cognitive Neuroscience (51/11)

Title: Human mesostriatal response tracks motivational tendencies under naturalistic goal-conflict

Authors: *T. GONEN[1], E. SOREQ[2], E. ELDAR[3], E. BEN SIMON[2], G. RAZ[2], T. HENDLER[2]; [1]Dept. of Neurosurg. & FBC, TLVMC, Tel Aviv-Yafo, Israel; [2]Functional Brain Ctr., Tel Aviv Med. Ctr., Tel Aviv, Israel; [3]Wellcome Trust Ctr. for Neuroimaging, Univ. Col. London, London, United Kingdom

Abstract: Goal-conflict situations, involving the simultaneous presence of reward and punishment, occur commonly in real life, and are known to unfold rapidly within our ever-changing environment. Such conflicts increase the complexity of motivational decision making and reflect well-known individual differences in the behavioral tendency to approach or avoid. However, despite accumulating neural depiction of motivational processing, the investigation of real life approach behavior and its interplay with individual tendencies is remarkably lacking. To that end, we developed a novel ecological interactive scenario which triggers motivational

behavior under high- or low- goal-conflict conditions. 55 healthy subjects played the game during an fMRI scan. Machine learning approach was applied to classify approach/avoidance behaviors during the game. To achieve an independent measure of individual tendencies, integrative profile was composed from three established theoretical models. Results demonstrated an extensive network underpinning dynamic approach behavior, including regions involved in incentive processing (e.g. Ventral Striatum [VS], Ventral Tegmental Area [VTA]), saliency tagging (e.g. Pulvinar and anterior Insula) and motor planning (e.g. supplementary motor area, premotor cortex). More specifically, approach under high-conflict involved activity confined to VTA, PAG, VS and precuneus. Notably, only VS and VTA activations during high-conflict discriminated between approach/avoidance personality profiles, suggesting that the relationship between integrative personality profile and naturalistic motivational tendencies is uniquely associated with the mesostriatal pathway. These findings are the first to unravel the multilevel relations between personality profile, real-time behavioral differences in approach tendencies and their underlying neural manifestation; thus enable new avenues for investigating approach-related psychopathologies.

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Poster

633. Neuroimaging of Reward Mechanisms

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Topic: G.02. Motivation

Support: DA015351

Title: Differential involvement of the prefrontal cortex and the amygdala in motivation

Authors: ***I. M. WHITE**[1,2], H. HOWARD[2], Z. ABBOTT[2], W. WHITE[1,2];
[1]Psychology, [2]Neurosci. Program, Psychology, Morehead State Univ., Morehead, KY

Abstract: Previously, we reported that the combination of stress and scopolamine severely impaired simple memory and that stress augmented scopolamine-induced memory deficits, producing a condition resembling that seen in the aged and in Alzheimer's patients. This study examined the involvement of the medial prefrontal cortex (mPFc) and the basolateral amygdala (BLA) in motivation and further examined the effects of scopolamine on motivation, using two appetitive tasks with differential workloads. Male Wistar rats were shaped to press a lever for a food reward, then received NMDA (10μg/μl, 0.5μl/site) or sham lesions in the mPFc or the BLA.

After recovery, rats were given alternating sessions of fixed ratio 5 (FR5) and fixed ratio 20 (FR20). The FR20 entailed a higher workload, and food procurement on the schedule required a higher level of motivation and more sustained attention. Prefrontal lesions markedly impaired performance on FR5 and FR20, with greater deficits during FR20, reflecting an impairment in motivation due to a greater workload requiring sustained attention. BLA lesions had little to no effect on FR5 but produced a slight impairment during FR20. Scopolamine-induced deficits were greater during FR20. Neither lesions nor scopolamine affected consummatory behavior. Our results suggest that the mPFc, but not the BLA, plays a role in motivation to procure food, and that the mPFc is necessary when a greater response requirement and sustained attention are in demand. Our results also suggest that the muscarinic receptor modulates prefrontal involvement in motivation, and they predict impaired motivation in Alzheimer's patients under conditions with a greater workload.

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Poster

633. Neuroimaging of Reward Mechanisms

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Title: Effects of behavioral activation on the neural basis of other perspective self referential processing in subthreshold depression: An fMRI study.

Authors: S. SHIOTA[1], Y. OKAMOTO[1], *G. OKADA[1], K. TAKAGAKI[1], M. TAKAMURA[1], A. MORI[1], S. YOKOYAMA[1], Y. NISHIYAMA[1], R. JINNIN[1], R. HASHIMOTO[2], S. YAMAWAKI[1];
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Abstract: It is known that subthreshold depression has a considerable impact on the quality of life, carries an elevated risk of developing a major depression, and can lead to serious functional impairment, including a negative impact on academic performance and social activity. Given

these concerns, it is very important to elucidate the pathogenic mechanisms underlying subthreshold depression and to develop effective interventions. It has been demonstrated that individuals with depression show negatively distorted evaluation of one's own self using his or her friend's viewpoint, and such altered self referential processing led to maintenance of depressive symptoms. Behavioral activation (BA) is one key component of cognitive behavior therapy for depression, and is an effective intervention for the treatment of major depression. However, brain mechanisms underlying the BA are not fully understood. We examined the effect of BA on neural activation during other perspective self referential processing in subthreshold depression. Freshmen attending Hiroshima University were recruited for the randomized controlled trial. Fifty-nine subjects underwent fMRI scans during a referential task with two viewpoints (self / other) and two emotional valences (positive / negative). For the intervention group, the scan occurred both before and after BA intervention, and the control group was also scanned twice, with an interval of 5 weeks without any intervention. We found an increased activation in the dorsal medial prefrontal cortex (dmPFC) in other perspective self reference for positive words after BA, and there was a positive correlation between the increased activation in the dmPFC and improvement of depressive symptoms. Additionally, reaction times for other perspective self referential judgement for positive words was increased in the intervention group after BA, and there was a positive correlation between improvement of depressive symptoms and the increased reaction times. BA increased dmPFC activation during other perspective self referential processing of positive words processing with improvement of depressive symptoms and increased reaction times which were associated with improvement of self monitoring function. These results suggest that BA led to increased activation in the dmPFC during other perspective self referential processing of positive words in people with subthreshold depression, which might contribute to improvement in depressive symptoms and objective monitoring function.

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Poster

633. Neuroimaging of Reward Mechanisms

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Topic: H.02. Human Cognition and Behavior

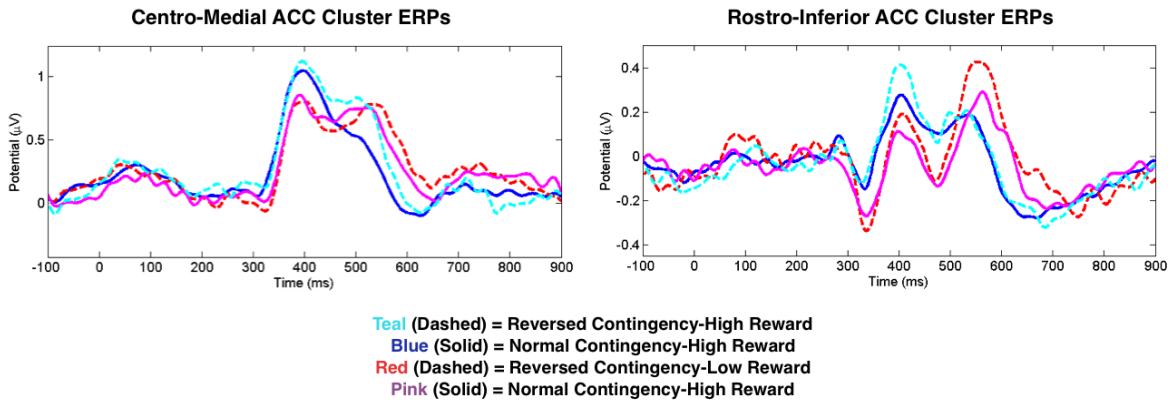
Support: NSF Grant: HSD SES-0527756

SLC Grant: SBE-0542013

Title: Reward processing and expectancy violation in dyadic-interaction: an EEG analysis

Authors: *K. JENSON[1], A. LI[2], S. MAKEIG[3], G. DEAK[2];
[1]UC San Diego, LA Jolla, CA; [2]Cognitive Sci., UC San Diego, La Jolla, CA; [3]Inst. for Neural Computation, La Jolla, CA

Abstract: To investigate real-time, naturalistic social decision-making, we collected EEG within a real-time, relatively unrestricted social game. Two subjects took part in each session (N = 26; 11 complete dyads). Subjects took turns touching one of two bubble animations shown on a screen between them with the goal of eliciting a "big pop" (high reward) instead of a "dull bloop" (low reward). Subjects were naïve to the feedback selection contingencies -- touching the same bubble that the other player had touched on the previous turn produces a pop -- and thus had to learn it by trial and error. Each session consisted of a 64-trial *rule-learning* block followed by two *unpredictable* blocks (totaling 400 trials) in which the outcome contingencies were reversed in 20% of the trials (randomly selected), and lastly a block of 48 trials with the original contingencies. Late Positive Complex ('P3') features of scalp ERPs were modulated by reward and uncertainty. We used independent component analysis (AMICA) to unmix the EEG signals, then clustered the resulting independent components (ICs) using k-means based on the equivalent dipole location. We further analyzed two IC clusters that accounted for the most ERP variance from 200 to 700ms. The equivalent dipole locations of ICs contributing to these two clusters were localized to different regions of anterior cingulate cortex (ACC): one centro-medial, the other rostro-inferior. ERP amplitudes for the more centro-medial-ACC cluster were larger for high-reward outcomes ($F(3,33) = 3.02$, $p = 0.04$) whereas for the more rostral-ACC cluster ERP amplitudes were larger following unexpected outcomes ($F(3,30) = 4.34$, $p = 0.01$). These results suggest that reward and uncertainty processing exhibit unique dynamics in distinguishable cortical networks. Further, these results provide additional evidence that the LPC/P3 consists of multiple spatially, temporally and functionally distinct source processes. Incidentally, the dynamics reported showed qualitatively similar, but non-significant, trends in trials in which participants viewed their partner's actions.



Disclosures: **K. Jenson:** None. **A. Li:** None. **S. Makeig:** None. **G. Deak:** None.

Poster

633. Neuroimaging of Reward Mechanisms

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Topic: G.02. Motivation

Title: Feasibility of multiband EPI for detection of brain activation in the reward system

Authors: *M. TAGHIZADEH SARABI, R. KEERATIVITTAYAYUT, K. NAKAHARA, R. AOKI;
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Abstract: The recently-advanced multiband echo planar imaging (MB-EPI) technique has become increasingly used in functional magnetic resonance imaging (fMRI) studies. In this study to examine whether MB-EPI can detect brain activation as satisfactorily as standard EPI (ST-EPI) in the reward system, we scanned twenty-four healthy human participants (twelve females and twelve males; mean age = 20.0 years, range = 18-23 years) using both MB-EPI (multiband factor = 5, TR = 720 ms, voxel size = 3 mm^3, TE = 30 ms, slice number = 45, flip angel = 52°) and ST-EPI (TR = 2500 ms, voxel size = 3 mm^3, TE = 30 ms, slice number = 38, flip angel = 80°). Participants took part in two fMRI sessions, one with MB-EPI and the other with ST-EPI, separated by five-minute break. The order of the sessions was counterbalanced across participants. In each fMRI session, participants performed 100 trials (divided into two runs) of the monetary incentive delay (MID) task. The task included five reward cue conditions (0 yen, ±20 yen, and ±400 yen), and the outcomes were defined as either hits or misses based on the participant's reaction time to a visual target presented after the cue. To identify brain activation to reward anticipation, we contrasted the hemodynamic response to the high-gain (+400 yen) vs. low-gain (+20 yen) cue. To identify brain activation to reward outcome, we contrasted hit vs. miss outcomes in the gain (+20 or +400 yen) trials. In the reward anticipation phase, we observed significant activation in the ventral striatum both in ST-EPI and MB-EPI sessions (one-sample *t* test, $P < 0.05$, FWE corrected across the whole brain), with no significant difference between the sessions (paired *t* test, $P > 0.05$, FWE corrected within the anatomical mask of the striatum). Likewise in the reward outcome phase, both ST-EPI and MB-EPI showed significant activation (one-sample *t* test, $P < 0.05$, FWE corrected across the whole brain) in the ventromedial prefrontal cortex (vmPFC), with no significant difference between the sessions (paired *t* test, $P > 0.05$, FWE corrected within the anatomical mask of the vmPFC). The results of the current study demonstrate that MB-EPI is as useful as ST-EPI in detection of brain activation in the striatum and vmPFC: two predominant brain regions of interest related to reward anticipation and outcome phase, respectively. Given the broader spatial coverage of the brain and higher temporal resolution, MB-EPI is a promising option to study the functions of the reward system as well as its interactions with other brain regions. The advantages of MB-EPI

will be further enhanced by selecting optimal scan parameters (TR, voxel size, and multiband factor), which should be determined in future studies.

Disclosures: **M. Taghizadeh Sarabi:** None. **R. Keerativittayayut:** None. **K. Nakahara:** None. **R. Aoki:** None.

Poster

634. Anxiety Disorders: Preclinical Models

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Topic: G.05. Anxiety Disorders

Support: NIH Grant AA019454

Title: Bidirectional modulation of aversive fear learning by 5-HT1A receptors in the BNST

Authors: ***C. A. MARCINKIEWCZ**, C. DORRIER, T. L. KASH;
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Abstract: Serotonin is often hailed as a positive regulator of emotion, but it also has a dark side. Selective serotonin reuptake inhibitors (SSRIs) often induce a negative emotional state characterized by feelings of anxiety and panic. The circuits through 5-HT modulates which these aversive states have not been defined, but converging evidence has implicated the bed nucleus of the stria terminalis (BNST) as a critical neural substrate. Recent work in our lab suggests that 5-HT_{2C} receptors in the BNST increase excitability of a subset of CRF neurons that enhance cued fear recall, but that these actions are opposed by 5-HT_{1A} receptors. In order elucidate the role of 5-HT_{1A} receptors in fear-related behavior, we utilized a transgenic floxed 5-HT_{1A} receptor mouse to genetically delete 5-HT_{1A} receptors in the BNST. Specifically, viral vectors containing the gene for Cre recombinase or a control vector were infused into the BNST of genetically modified 5-HT_{1A} receptor mice. Using a classic tone/shock fear conditioning paradigm, we then assessed fear learning and recall. Our preliminary results indicate that genetic deletion of 5-HT_{1A} receptors in the BNST reduces cued fear but enhances contextual fear recall. These data suggest that associative fear learning may be bidirectionally modulated by 5-HT_{1A} and 5-HT_{2C} receptors in the BNST.

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Poster

634. Anxiety Disorders: Preclinical Models

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Topic: G.05. Anxiety Disorders

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Title: JNK1 regulates adult hippocampal neurogenesis and controls anxiety and depressive behaviour

Authors: *F. MARCHISELLA[1], H. MOHAMMAD[1], S. ORTEGA-MARTINEZ[1], P. HOLLOS[1], K. EEROLA[2], E. KOMULAINEN[1], N. KULESSKAYA[3], E. SAVONTAUS[2], H. RAUVALA[3], B. PETERSON[4], H. VAN PRAAG[4], E. COFFEY[1];
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Abstract: Anxiety and depression together represent the second largest cause of years lived with disability (YLD) (Vos et al. 2012), and the World Health Organization projects that by 2020, neuropsychiatric conditions will account for 15 % of YLD worldwide. Depression and anxiety show comorbidity of up to 60 %, suggesting that there is overlap in the underlying pathophysiological mechanism. Sad mood and lack of pleasure in daily situations (anhedonia) are common features of depression, while an excessive and overwhelming sense of worry and anticipation of fear are common features of anxiety.
It is widely reported in the literature that the stimulation of adult hippocampal neurogenesis, is required for the therapeutic effect of different classes of anti-depressant drugs: serotonin reuptake inhibitors, selective norepinephrine reuptake inhibitors and electroconvulsive shock (Duman 2004). Conversely, decreased neurogenesis is associated with anxiety and depression in mice (Snyder et al. 2011) and in humans (Drew & Hen 2007). Hence, targeting mechanisms that regulate adult hippocampal neurogenesis is expected to induce neuroplasticity changes that improve mood and alleviate anxiety and depression.
c-Jun N-terminal kinases (JNKs) are members of the mitogen-activated protein kinase (MAPK) family that regulate physiological and pathological processes in the central nervous system (Coffey 2014). In particular, JNK1 is a dominant regulator of neuronal differentiation during development. Here, we investigate the role of JNK1 in adult hippocampal neurogenesis using knockout mice and inhibitor infusion. Histological analysis of brain sections from these animals display significant changes in hippocampal neurogenesis, dentate gyrus volume and dendritic

morphology upon genetic ablation of *Jnk*1, as does infusion with a specific inhibitor of JNK. We will present data from a battery of behavioral tests that measure anxiety and depression-related behavior in mice where JNK signaling is disturbed following genetic ablation, pharmacological inhibition or retroviral targeting of neurogenic niche cells.

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Poster

634. Anxiety Disorders: Preclinical Models

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Topic: G.05. Anxiety Disorders

Support: NIH Grant MH106568

Title: Elucidating the neural circuitry of social familiarity-induced anxiolysis.

Authors: S. MAJUMDAR[1], E. A. LUNGWITZ[1], R. DU[2], K. D. ANDREWS[3], A. D. DIETRICH[4], *W. A. TRUITT[4];
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Abstract: The social interaction habituation (SI-hab) protocol has been used to demonstrate that rats acquire social familiarity-induced anxiolysis (SoFiA), which is a type of safety learning where rats display reduced anxiety-like behavior (anxiolysis) in the presence of a familiar partner. SoFiA acquisition is dependent on both anxiogenic stimulus and social familiarity during training sessions. Additionally, SoFiA acquisition and expression are dependent on the ventro-medial prefrontal cortex (vmPFC). *Thus SoFiA acquisition and expression can be simplified into four different constructs: social memory, anxiety, safety learning and anxiolysis and these constructs are hypothesized to be coordinated by the vmPFC.* However, the SoFiA neural circuitry has not been identified. As a first step towards elucidating the SoFiA neural circuitry we are identifying key neural structures with activity patterns corresponding to each of the SoFiA constructs. The SoFiA constructs can be isolated, by manipulating the SI-hab protocol. Briefly, the SI-hab protocol involves a baseline SI test followed by 5 daily social training sessions, which are comprised of a 5 min social interaction test. Two main factors during the social training sessions were manipulated, type of conspecific (familiar or novel) and anxiogenic stimulus (low vs high). The anxiogenic stimuli for these studies were control (dim

light) for low and the bright light challenge (BLC) for high anxiety stimulus. Anxiety-like behavior was determined by social interaction (SI) time, time experimental rat spent initiating social investigation, where SI time is inversely related to anxiety-like behavior. This resulted in four groups: 1. Control (low anxiety + novel partner); 2. Social memory (low anxiety + familiar partner); 3. Anxiety (high anxiety + novel partner); 4. SoFiA (high anxiety + familiar partner). As expected, for groups 1 & 2 SI time remained similar to baseline across all SI-hab sessions, indicating no change in anxiety-like behavior. Compared to baseline, group 3 displayed reduced SI time during all SI-hab sessions. Group 4 displayed a transient reduction in SI time in the initial SI-hab sessions, compared to baseline, and returned to baseline SI times by day5 indicating a SoFiA response. For experiment 1, rats were perfused 90 min following the last SI-hab session and brains were processed for cFos immunohistochemistry. Rats for experiment 2 were sacrificed and brains were processed for RNA isolation for immediate early gene expression (qPCR). From these tissues we are in the process of identifying brain regions with immediate early gene expression that correspond with the 4 treatment conditions.

Disclosures: S. Majumdar: None. **E.A. Lungwitz:** None. **R. Du:** None. **K.D. Andrews:** None. **A.D. Dietrich:** None. **W.A. Truitt:** None.

Poster

634. Anxiety Disorders: Preclinical Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 634.04/FFF23

Topic: G.05. Anxiety Disorders

Support: Janeway Foundation

Memorial University Dean of Science

NSERC-USRA

Title: Increased anxiety and altered metabolic set-point in a juvenile mouse model of chemotherapy-induced alopecia

Authors: *M. D. BERRY[1], D. GARDINER[1], M. C. ENNIS[1,2], H. M. RIVKIN[1], P. E. MCCALLUM[2], M. S. PITTS[1,2], J. BLUNDELL[2];
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Abstract: Growing evidence indicates that pharmacotherapy of cancer can be associated with an increased incidence of anxiety, depression, and cognitive impairments that persist long after

chemotherapy has concluded. In the current study we examine for the presence of behavioural markers of such deficits in our previously established model of chemotherapy-induced alopecia in juvenile, female C57BL/6 mice, and determine whether the known neuroprotectant, (-)deprenyl, can prevent such changes. All animals were housed on a reverse light cycle with food and water *ad libitum*. Mice were depilated at 5-6 weeks of age and cyclophosphamide (150 mg/kg i.p.) administered on days 7, 9, and 11 post-depilation. On day 7 post-depilation animals also had an osmotic minipump implanted intraperitoneally for delivery of either (-)deprenyl (0.01 - 1 mg/kg/day) or vehicle. At various time points post-cyclophosphamide animals were tested for anxiety (elevated plus maze), depression (forced swim) and cognition (fear memory) with euthanasia at post-depilation day 35. Weight change, and food and water consumption was monitored daily. A pronounced anorectic effect of cyclophosphamide administration was observed ($P < 0.001$) which lasted for the duration of the study. Daily administration of (-)deprenyl dose-dependently normalized weight gain. In contrast, daily intake of food and water was not altered by any of the treatments. Weight loss in the presence of normal food intake suggests a change in metabolic set-point in response to cyclophosphamide that is prevented by concurrent low dose (-)deprenyl. Although general locomotor activity of animals was not altered, cyclophosphamide administration was associated with a pronounced anxiogenic effect, with treated animals spending significantly less time in the open arms of the elevated plus maze ($P < 0.01$). This effect was also prevented in a dose-dependent manner by (-)deprenyl. In contrast, no evidence of cognitive decline was observed when tested one week following the last dose of cyclophosphamide. Forced swim data is currently being analyzed. In conclusion, at doses that induce alopecia, cyclophosphamide treatment also induces an anorectic and anxiety phenotype in juvenile, female, C57BL/6 mice consistent with clinical reports. This can be prevented by low dose co-administration of the neuroprotectant (-)deprenyl. As such (-)deprenyl may be a useful adjunct to prevent the side effects associated with cancer chemotherapy.

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Poster

634. Anxiety Disorders: Preclinical Models

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Topic: G.05. Anxiety Disorders

Support: TRDRP Award 24RT-0023

Title: The role of endogenous orphanin FQ/nociceptin in anxiety-like behaviors and rewarding actions of alcohol

Authors: *S. V. PEREZ[1], P. MARQUEZ[2], A. HAMID[2], A. HAMID[2], O. FARHAD[2], K. LUTFY[2];
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Abstract: Endogenous orphanin FQ/nociceptin (OFQ/N) has been implicated in anxiety and the rewarding action of alcohol. However, the role of sex in this regard has not been studied previously. Thus, one of the goals of the current study was to determine if the level of anxiety or the magnitude of alcohol-induced conditioned place preference (CPP) would be different between mice lacking the prepro-OFQ/N (ppOFQ/N) and their wild-type controls, and if there would be a sex-related difference in this response. Considering that anxiety may be one of the leading causes of alcohol use disorder and alcoholism, we also examined if exposure to the elevated plus maze (EPM), that is widely used as a model of fear and anxiety, would alter the rewarding action of alcohol and whether there is a sex-related difference in this regard. Male and female mice lacking the ppOFQ/N gene and their wild-type controls were either tested for anxiety-like behaviors using the EPM test (exposed group) or left undisturbed (not-exposed group). The following week mice were tested for alcohol-induced CPP, in which mice were tested for baseline place preference on day 1, conditioned with saline/alcohol (2 g/kg) or alcohol/saline twice daily (15 min each session) on days 2-4 and then tested for postconditioning place preference on day 5. Our results showed that male but not female mice lacking the ppOFQ/N gene spent lesser time on the open arms of the EPM compared to their wild-type littermates, suggesting that null mice exhibited more anxiety-like behaviors compared to their wild-type controls and there was a sex-related difference in this regard. While there was no difference in the magnitude of ethanol-induced CPP between non-exposed mice lacking the ppOFQ/N gene and their wild-type controls, there seemed to be a greater CPP response in female mice compared to male wild-type mice but this difference was not observed between male and female mice lacking the ppOFQ/N gene. Interestingly, male but not female mice lacking the ppOFQ/N exposed to the EPM exhibited a significantly greater CPP response compared to their wild-type controls. Together, the results of the present study show that the rewarding action of alcohol was enhanced in animals exhibiting greater anxiety and endogenous OFQ/N system may serve a protective role in this regard, and there may be a sex-related difference in this regard.

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Poster

634. Anxiety Disorders: Preclinical Models

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Topic: G.05. Anxiety Disorders

Support: CNRM 60855-300600-7.01

Title: Effects of repeated testing on anxiety-like behaviors in the elevated plus maze and elevated zero maze in male and female C57BL/6J mice

Authors: *L. B. TUCKER[1,2], A. H. FU[1,2], J. T. MCCABE[1,2];
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Abstract: The elevated plus maze (EPM) and elevated zero maze (EZM) are behavioral tests that are widely employed to assess anxiety-like behaviors in rats and mice following experimental manipulations, or to test the effects of pharmaceutical agents. Both of these tests are based on approach/avoidance conflict, with rodents perceived as “less anxious” being more willing to explore the brighter, open and elevated regions of the apparatus as opposed to remaining in the darkened and enclosed regions. There are experimental designs in which there may be a need to measure anxiety at multiple time points following an experimental manipulation, but there is a paucity of data regarding the effects of repeated testing in these apparatuses. In this experiment, male and female C57BL/6J mice were tested either daily or weekly in the EPM or EZM for a total of five exposures. Preliminary data suggest that mice tend to be more anxious in the EPM than in the EZM, as measured by the amount of time spent in the anxiogenic zones of the apparatus. Mice were more likely to habituate to the EPM than to the EZM with either daily or weekly testing, as both the total distance traveled and amount of time in the open arms of the EPM decreased with more exposures. These measures remained relatively stable in the EZM. There was a drastic effect of cohort on anxiety-like behaviors, which may have resulted from a change in housing rooms for one group of animals the day prior to the beginning of testing. However, this only had an effect on behavior in the EPM, suggesting that behavior in the EZM may be more resistant to the effects of environmental stressors. These preliminary data do not suggest any overt sex differences. In summary, results at this time indicate that the design of the EZM encourages greater exploration of anxiogenic regions in mice, and the lack of habituation to the apparatus makes the EZM more suitable for experimental designs in which repeated testing is necessary.

Disclosures: L.B. Tucker: None. **A.H. Fu:** None. **J.T. McCabe:** None.

Poster

634. Anxiety Disorders: Preclinical Models

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Topic: G.05. Anxiety Disorders

Support: USC ASPIRE II

VA Merit 1I01BX001374

Title: Exploring the role of orexinergic mechanisms in mediating individual differences in fear extinction responses.

Authors: *A. C. SHARKO, K. F. KAIGLER, J. R. FADEL, M. A. WILSON;
Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: Post-traumatic stress disorder (PTSD) is a severe anxiety disorder that can develop after experiencing a life-threatening trauma, such as combat service, assault, or natural disaster. Not everyone who experiences these types of traumas develops PTSD, suggesting that some neurobiological factors may confer resiliency, or risk, to the long-term negative effects of traumatic stress. Our laboratory has demonstrated that outbred Long-Evans rats show individual differences in conditioned anxiety-like behaviors, with significant variability in extinction of freezing behavior following fear conditioning, suggesting that this strain may serve as a useful model for characterizing the neurobiological mechanisms that underlie differential sensitivity to traumatic stress. The orexin/hypocretin system, in particular, has been implicated in the modulation of stress-induced anxiety-like behaviors and conditioned fear responses. Hypothalamic orexin neurons project to the prefrontal cortex and the amygdala, two brain regions known to mediate behavioral responses to stressful stimuli. Orexin neurons also project directly to cholinergic neurons in the basal forebrain, which modulate attentional processes through inputs to the prefrontal cortex and amygdala. Using dual label immunohistochemistry, Fos expression in orexinergic neurons was assessed to identify differential patterns of activation in these neuronal populations associated with individual differences in the extinction of fear-conditioned freezing behaviors. Two cohorts of rats were exposed to three tone-shock pairings, followed by extinction training two days later in a novel environment with twenty cue (tone) presentations. Rats were divided into high or low extinction groups based on freezing behavior during the last ten minutes of this extinction trial. Extinction learning was assessed two days later and tissue was collected for immunohistochemical analysis. Rats showing resistance to fear extinction (high freezers) had significantly more dual Fos/orexin-A positive cell counts in the medial hypothalamus than low freezers, suggesting that differential activation of orexin neurons may contribute to individual differences in extinction of fear responses. Interestingly, no individual differences were detected in the lateral hypothalamus. Additional studies examining

the behavioral and neurobiological effects of pharmacological manipulation of orexinergic signaling suggest that orexin activity may modulate consolidation of conditioned fear memories or expression of fear conditioned behaviors. Support: USC ASPIRE II funding to ACS and VA Merit Award 1I01BX001374 to MAW

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Poster

634. Anxiety Disorders: Preclinical Models

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Title: Neuronal deletion of Kmt2a/Mll1 histone methyltransferase in ventral striatum is associated with defective spike-timing dependent striatal synaptic plasticity, altered response to dopaminergic drugs and increased anxiety

Authors: *B. JAVIDFAR[1], E. Y. SHEN[2], Y. JIANG[2], B. KASSIM[2], Y.-H. E. LOH[3], Q. MA[4], A. C. MITCHELL[2], V. POTHULA[1], A. STEWART[5], P. ERNST[6], W.-D. YAO[4], G. E. MARTIN[7], L. SHEN[3], M. JAKOVCEVSKI[8], S. AKBARIAN[2];
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Abstract: *Lysine methyltransferase 2a (Kmt2a)* and other regulators of H3 lysine 4 methylation, a histone modification enriched at promoters and enhancers, are widely expressed throughout the brain but molecular and cellular phenotypes in subcortical areas remain poorly explored. We report that *Kmt2a* conditional deletion in postnatal forebrain is associated with excessive

nocturnal activity and paradoxical or blunted responses to stimulant and dopaminergic agonist drugs, in conjunction with near-complete loss of spike timing-dependent long term-potentiation (tLTP) in medium spiny neurons. Selective ablation of *Kmt2a,* but not the ortholog *Kmt2b*, in adult ventral striatum/nucleus accumbens neurons markedly increased anxiety scores in multiple behavioral paradigms. Striatal transcriptome sequencing in adult mutants identified 262 *Kmt2a*-sensitive genes, mostly downregulated in *Kmt2a* deficient mice. Transcriptional repression includes the *5-Htr2a* serotonin receptor, strongly associated with anxiety and depression related disorders in human and animal models. Consistent with the role of *Kmt2a* in active transcription, the transcriptional regulators *Isl1* and *Sp9* were downregulated and affected by H3K4 promoter hypomethylation. Therefore, *Kmt2a* regulates synaptic plasticity in striatal neurons and provides an epigenetic drug target for anxiety and dopamine-mediated behaviors.

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Poster

634. Anxiety Disorders: Preclinical Models

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Program#/Poster#: 634.09/GGG2

Topic: G.05. Anxiety Disorders

Support: Department of Psychology at Ithaca College

Title: Effects of environment on behaviors in the elevated plus maze, marble arena, and hole board in the neoclomipramine rodent model of Obsessive Compulsive Disorder

Authors: *S. R. LOCKHART[1], D. D. MARINARO[1], D. S. KREISS[1,2];
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Abstract: Obsessive Compulsive Disorder (OCD) is characterized by persistent, anxiety producing thoughts accompanied by overwhelming urges to perform repetitive, ritualistic behaviors. Modern pharmacological treatments for OCD are only effective in 40-60% of patients and have an 8-10 week delayed onset. Establishment of an animal model of this debilitating disorder would provide an invaluable research tool for the development of new avenues of treatment. The current study evaluated environmental effects on an animal model of OCD based upon neonatal exposure to clomipramine, an uptake inhibitor of serotonin and norepinephrine. In

this model, experimental male Sprague-Dawley rats are administered 15 mg/kg clomipramine twice daily during neonatal Days 9-16 (= neoclom). Prior studies have demonstrated that the neoclomipramine model has both face and predictive validity assessed via the Hole Board, Marble Arena, and Elevated Plus Maze (SFN and FUN abstracts, 2013, 2014, 2015). To investigate the influence of diverse environmental conditions upon this OCD model, the present study quantified the behaviors of neoclom and control rats following long term housing in either an enriched or an isolated environment. **Results showed that neonatal exposure to clomipramine attenuated the effects of being housed in an ISOLATED environment.** Control animals reared in an ENRICHED environment spent significantly more time in open arms of EPM, less time in closed arms, and had more open arm entries and total arm entries as compared to control rats reared in ISOLATION. In the Hole Board, control rats reared in an ENRICHED environment trended toward poking less than control ISOLATED rats (p=0.057). However, neoclom ENRICHED rats only differed from neoclom ISOLATED rats in the number of pokes per hole. **Results also indicated that being housed in isolation noticeably attenuated the effects of neonatal exposure to CLOMIPRAMINE.** Compared to enriched CONTROL rats, enriched rats neonatally exposed to CLOMIPRAMINE spent significantly more time in the closed arms and had trends for less time spent in the open arms (p=0.072), less open arm entries (p=0.061), less total arm entries (p=0.065) and more pokes (p=0.063). In contrast, the only behavioral difference exhibited by isolated NEOCLOM rats versus isolated CONTROL animals was a trend for more time spent in closed arms of the EPM (p=0.070). Taken together, the present study emphasizes the importance of environmental conditions when evaluating the validity of behavioral animal models of OCD and other anxiety disorders.

Disclosures: S.R. Lockhart: None. **D.D. Marinaro:** None. **D.S. Kreiss:** None.

Poster

634. Anxiety Disorders: Preclinical Models

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Topic: G.05. Anxiety Disorders

Title: Stress related alterations in neurotransmitter levels in the prefrontal cortex and amygdala; an *In vivo* microdialysis study

Authors: M. MONBUREAU, A. ARANOV, H. KOOIJKER, Y. CHANG, L. YU, C. CIARELLO, M. VAN DER HART, N. MOORE, H. JANSSENS, *A. RASSOULPOUR; Brains On-Line, LLC, South San Francisco, CA

Abstract: It has been previously demonstrated that exposure to various stressors can modulate amino acids and monoamines in different brain regions. Social defeat stress has been shown to modulate dopamine (DA) levels in the basal ganglia and cortex (Tidely and Miczek, 1996), restraint stress was shown to modulate levels glutamate (Glu) and aspartate levels in the amygdala (Reznikov; 2007), cortex and basal ganglia (Moghaddam, 2006), and psychological stress was shown to modulate serotonin (5HT) levels in the amygdala and cortex (Kawahara et al, 1993). Based on some of these findings, we set out to do a side by side comparison of different stress models (chronic social defeat, psychological stress induced by foot shock, and restraint stress) on changes in a panel of neurotransmitters (NT) in the brain of freely moving rats. To this end, bi-lateral microdialysis probes were placed in the prefrontal cortex and amygdala of adult rats and the levels of DA, 5HT, Glu, norepinephrine (NE), gamma-aminobutyric acid (GABA), and glycine (gly) were measured before, during and after exposure to various stressors. We found regionally distinct changes within animals that were paradigm dependent. For example, increases in DA, 5HT, and NE were observed in the amygdala of rats exposed to foot shock while socially defeated animals showed an increase in cortical levels of DA, 5HT, NE, and GABA when exposed to aggressive animals. The current findings confirm and expand on previous published results on the ability of various stressors to modulate NT levels in different brain regions. We have been able to demonstrate that various brain regions and NT systems are differentially involved in the stress response.

Disclosures: M. Monbureau: None. **A. Aranov:** None. **H. Kooijker:** None. **Y. Chang:** None. **L. Yu:** None. **C. Ciarello:** None. **M. van der Hart:** None. **N. Moore:** None. **H. Janssens:** None. **A. Rassoulpour:** None.

Poster

634. Anxiety Disorders: Preclinical Models

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Topic: G.05. Anxiety Disorders

Support: DFG CRC-TRR-58 grants Z02 and A09

Title: GLRB spastic mice reveal abnormal open space behavior

Authors: *N. SCHAEFER[1], C. RÜDT VON COLLENBERG[1], B. WACHTER[1], R. BLUM[1], J. DECKERT[2], C. VILLMANN[1];
[1]Inst. For Clin. Neurobio., Wuerzburg, Germany; [2]Dept. of Psychiatry, Psychosomatics and Psychotherapy, Wuerzburg, Germany

Abstract: *GLRB* encodes the β-subunit of the adult glycine receptor (GlyR). This inhibitory ion channel is important for fast inhibitory neurotransmission in the central nervous system. Glycine receptors form a pentameric receptor complex of two α and three β subunits. The β subunit is required for GlyR anchoring via gephyrin to the postsynaptic cytoskeleton. So far, mutations in the *GLRB* gene have been assigned to the neuromotor disorder startle disease. Patients with this rare neurological disorder suffer from exaggerated startle responses following unexpected noise or tactile stimuli.
A genome-wide association study provided evidence for a link between *GLRB* gene polymorphisms of agoraphobic (AG) patients with an anxiety phenotype based on an Agoraphobic Cognitions Questionnaire (ACQ) in healthy German volunteers. AG is characterized by abnormal open space behavior as well as distorted cognitive processes. Familial aggregation has been determined for AG and PD (panic disorder). Twin studies exhibited a respective heritability in PD and AG of about 38%-48%.
To further analyze the possible cause of AG/PD phenotype by mutations within the *GLRB* gene, we performed an analysis of agoraphobic behavior in mice with a partial *Glrb* knock-out (*spastic*). So far, *spastic* mice resemble the best characterized *Glrb* mouse model. Homozygous *spastic* mice show a neuromotor phenotype due to a splice defect within the *Glrb* gene. The splice defect generates aberrant splice products that lack exon 6 or a combination of exons 5 and 6. As a consequence, reduced amounts of full-length GlyR β protein have been observed. Heterozygous *Glrb* mutants lack about 50% of full-length GlyRβ in brain regions including cortex, cerebellum, thalamus, striatum, hippocampus, brain stem and spinal cord. Notably, heterozygous *spastic* mice show a quite inconspicuous behavioral phenotype, beside an avoidance of a novel open space, a behavior we recently confirmed to relate to agoraphobic fear in humans.
In summary, our findings provide evidence that *GLRB* allelic variation may contribute not only to the neurological disorder hyperekplexia, but also to the risk of the categorical anxiety disorder PD/AG by increasing startle response and agoraphobic behavior.

Disclosures: **N. Schaefer:** None. **C. Rüdt von Collenberg:** None. **B. Wachter:** None. **R. Blum:** None. **J. Deckert:** None. **C. Villmann:** None.

Poster

634. Anxiety Disorders: Preclinical Models

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Topic: G.05. Anxiety Disorders

Support: NSC 101-2320-B-010 -041 -MY3

Title: FKBP51 depletion upregulates HTR1A and ameliorates BDNF reduction and anxiety-like behavior in mice under chronic stress

Authors: R.-H. HE[1], C.-Y. WANG[3], *Y.-H. LEE[2];
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Abstract: FK506 binding protein 51 (FKBP51) encoded by *Fkbp5* gene is a negative feedback co-chaperon of glucocorticoid receptor, and is upregulated in the brain under chronic stress. Overexpression of FKBP51 has been implicated in the drug resistance in patients with depression and bipolar disorders. 5-HT_{1A} receptor (HTR1A) and BDNF are the two major mental state mediators downregulated in stress-induced depression, but whether their expressions are regulated by FKBP51 were unexplored. In the present study, we examined the role of FKBP51 in the expression of HTR1A and anxiety-like behavior used 4-day dexamethasone (Dex) intake-induced stress mouse model. We found that 4-day Dex intake induced anxiety-like behavior in the elevated plus maze test but not depression-like behavior in the sucrose preference test. This behavioral phenotype was attenuated in Fkbp5-knockout (FKBP51KO) mice. The protein and mRNA levels of FKBP51 were elevated whereas BDNF were decreased under stress, and the latter was significantly upregulated in FKBP51KO mice. Interestingly, HTR1A expression was not affected under chronic stress in wild-type mice, but become much higher in FKBP51KO mice under stress. Together, the results suggest that FKBP51 plays important roles in the expresion of $5HT_{1A}$ receptor and BDNF in stress-induced anxiety, which provide important clues for the therapeutic strategy of stress-induced neuropsychiatric disorders.

Disclosures: R. He: None. **C. Wang:** None. **Y. Lee:** None.

Poster

634. Anxiety Disorders: Preclinical Models

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Program#/Poster#: 634.13/GGG6

Topic: G.05. Anxiety Disorders

Title: Behavioral profiles of C57BL/6 and C57BL/10 mouse substrains: implications for genetic engineering studies

Authors: *C. ST. PIERRE[1], N. M. GONZALES[2], A. A. PALMER[1];
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Abstract: C57BL/6 (B6) mice are the most widely used inbred strains in biomedical research. Several B6 substrains exist that have been maintained as isolated breeding populations for

several decades. These B6 substrains are known to exhibit numerous behavioral and genetic differences. C57BL/10 (B10) mice have also given rise to a number of substrains, which are most commonly used for immunological rather than behavioral studies. We subjected a panel of thirteen different B6 and B10 substrains to a battery of behavioral tests relevant to addiction and psychiatric disorders. The panel included eight C57BL/6 strains (C57BL/6J, C57BL/6NJ, C57BL/6JBomTac, C57BL/6NTac, B6N^{-TyrC}/BrdCrlCrl, C57BL/6NCrl, C57BL/6ByJ, and C57BL/6NHsd) and five C57BL/10 strains (C57BL/10J, C57BL/10ScCr, C57BL/10ScSnJ, C57BL/10SnJ, and C57BL/10ScNHsd). We found significant behavioral differences among the substrains in the open field test, locomotor response to cocaine, fear conditioning, prepulse inhibition, and forced swim test. Our results suggest these substrains have characteristic behavioral profiles that can present a challenge or opportunity for studying mutated genes of interest in disease-relevant CNS pathways. The genetic background should be carefully considered when selecting a substrain in order to detect the predicted behavioral phenotypes and interpret them appropriately. This approach may greatly facilitate the elucidation of candidate genes underlying behavioral differences in psychiatric disorders. We are currently conducting gene expression analyses in several tissues for each substrain in an effort to further understand their behavioral differences.

Disclosures: **C. St. Pierre:** None. **N.M. Gonzales:** None. **A.A. Palmer:** None.

Poster

634. Anxiety Disorders: Preclinical Models

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Topic: G.05. Anxiety Disorders

Support: Chang Gung Hospital CMRPD1F0091

Title: Effects of NPTX2 on anxiety related behaviours in mice

Authors: *G.-J. HUANG[1], S. CHANG[2];
[1]Chang Gung Univ., Taoyuan City, Taiwan; [2]Chang Gung Univ., Taoyuan city, Taiwan

Abstract: Anxiety disorders can have a significant impact on quality of life and exacerbate other psychiatric conditions associated with high morbidity and mortality such as depression. In our previous study, we used chronic exercise or antidepressants to alter animal anxiety and depression-like behaviours. We identified a list of candidate genes in the hippocampus for which changes in expression were associated with these interventions. Among these genes, we hypothesize that neuronal pentraxin 2 (NPTX2) plays an important role in anxiety. To confirm

this, we specifically knocked out this gene in the central nervous system to by crossing *Nptx2* floxed mice with Sox1-Cre mice. Interestingly, *Nptx2* KO mice exhibited higher levels of anxiety compared to WT mice. In addition, we revealed that *Nptx2* KO mice feature decreased neurogenesis and dendritic spine density together with higher HPA axis and neuronal activity following acute stress. Next, to rule out the possibility that the anxiety phenotype in *Nptx2* KO mice was due to impaired neurodevelopment, we crossed the *Nptx2* floxed mice with CAG-CreER mice to generate an inducible KO model. Our data indicated that inducible *Nptx2* KO mice featured similar deficits. In conclusion, we demonstrated that *Nptx2* influences anxiety behaviours, neurogenesis and neuronal activities.

Disclosures: G. Huang: None. **S. Chang:** None.

Poster

634. Anxiety Disorders: Preclinical Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 634.15/GGG8

Topic: G.05. Anxiety Disorders

Support: TIFR Intramural Grant

LTMT Grant

Title: Forebrain Sirt1 regulates anxiety-like behavior

Authors: *S. GHOSH, S. MUKHOPADHYAY, S. OLKAR, U. SEETHARAM-KOLTHUR, V. A. VAIDYA;
Tata Inst. of Fundamental Res., Mumbai, India

Abstract: Sirt1 is a NAD+ dependent protein deacetylase which acts as a metabolic sensor within several tissues. Sirt1 is highly expressed in the mammalian brain, including limbic circuits such as the hippocampus and prefrontal cortex. Multiple studies have shown that Sirt1 can regulate neuronal survival and differentiation, expression of trophic factors, and synaptic plasticity within these circuits. Previous reports using brain specific Sirt1 knockout mouse lines have implicated neuronal Sirt1 in the regulation of anxiety and depression-like behavior. However, the specific brain regions via which Sirt1 exerts its effects on mood remain poorly characterized. In our study, we have used a forebrain-specific Sirt1 knockout (Sirt1KO) mouse line, generated by crossing Emx1-Cre expressing mice to mice expressing an allele of Sirt1 in which exon 4, containing the catalytic unit, is flanked by loxP sites, to test whether selective removal of functional Sirt1 from the forebrain influences anxiety or depression-like behavior.

Sirt1KO mice exhibit normal development, and were tested on a battery of anxiety and depression-like behavioral tasks upon reaching adulthood. Our results indicate that Sirt1KO animals display reduced anxiety in the open field test, with increased time spent and increased distance moved in the center. Sirt1KO mice also show anxiolytic behavior in the elevated plus maze test, with increased time spent and increased distance moved in the open arms. In contrast, depression-like behavior, as assessed by the tail suspension and forced swim tests, was unchanged in Sirt1KO mice when compared to their wild type littermate controls. Enhanced anxiolysis observed in forebrain-specific Sirt1KO mice recapitulates the decline in anxiety behavior observed in whole-brain Sirt1 knockout mice. In contrast, while whole-brain Sirt1 knockout mice also exhibit antidepressant-like behaviors, forebrain-specific Sirt1KO mice do not show any change in depression-like behavior on behavioral despair tasks. Our studies support a role for forebrain cortical Sirt1 in the regulation of anxiety behavior. Experiments are currently underway to understand the specific regulation of Sirt1-responsive signaling pathways and molecular targets that may mechanistically mediate the behavioral changes observed in the forebrain-specific Sirt1KO mice.

Disclosures: **S. Ghosh:** None. **S. Mukhopadhyay:** None. **S. Olkar:** None. **U. Seetharam-Kolthur:** None. **V.A. Vaidya:** None.

Poster

634. Anxiety Disorders: Preclinical Models

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Program#/Poster#: 634.16/GGG9

Topic: G.05. Anxiety Disorders

Title: Functional role of cortical layer VIb in mouse behaviour

Authors: *L. GUIDI[1], K. V. KORRELL[1], A. HOERDER-SUABEDISSEN[1], P. L. OLIVER[1], M. C. WILSON[2], P. O. KANOLD[3], D. BANNERMAN[1], Z. MOLNAR[1];
[1]Univ. of Oxford, Oxford, United Kingdom; [2]Univ. of New Mexico, Albuquerque, NM; [3]Univ. of Maryland, College Park, MD

Abstract: Subplate neurons are amongst the earliest generated in the mammalian cerebral cortex and play a critical role during the development of cortical and thalamo-cortical circuits (Hoerder-Suabedissen A, Molnár Z, 2015 Nat Rev Neurosci 16:133-46). The majority of this diverse cell group undergoes cell death over the course of postnatal development - the remainder forms cortical layer VIb. Although impaired subplate is linked to neurodevelopmental disorders (Hoerder-Suabedissen A et al, 2013 PNAS, 110:3555), little is known about the functional roles of the adult layer VIb.

Here, we investigate putative functions of these cells by inhibiting evoked synaptic transmission in a subset of VIb neurons using a Drd1a-Cre line crossed with floxed Snap25 mice. We have found that the Drd1a+ layer VIb neurons project to higher order thalamic nuclei and hypothesise they are responsible for gating transthalamic communication of cortical areas. This raises the possibility that a small population of cortical neurons regulates a range of behaviours dependent on cortical function.
Layer VIb is the only cortical cell population that responds to orexin, a key neurotransmitter for sleep and arousal (Bayer et al, 2002 J Neurosci. 22:7835-9), which is also important in regulating emotional behaviour, particularly fear and anxiety (Flores A et al, 2015 Trends Neurosci 38:550-9). Based on this, we are investigating whether layer VIb plays a role in modulating behavioural performance in tasks associated to circadian activity, anxiety and fear. Our preliminary results reveal that silencing Drd1a+ layer VIb neurons does not affect circadian rhythms in normal and abnormal light/dark cycles but it had an anxiolytic effect on a range of behavioural tasks in a cohort of VIb mutants and wildtype controls (n = 20). We found that VIb "silenced" mice spent more time in open arms in the elevated plus maze ($p<0.001$), more time in the light area of the light-dark box ($p<0.05$) and also approached novel foods more quickly ($p<0.05$). We are currently investigating this system further by manipulating function of this largely uncharacterised cortical layer.
Determining the function of layer VIb neuronal populations could reveal a novel element in cortico-thalamic circuits and how cortical areas communicate. This circuit is known to be severely disrupted in neuropsychiatric disorders and may have a major influence on the mode of information processing.

Disclosures: **L. Guidi:** None. **K.V. Korrell:** None. **A. Hoerder-Suabedissen:** None. **P.L. Oliver:** None. **M.C. Wilson:** None. **P.O. Kanold:** None. **D. Bannerman:** None. **Z. Molnar:** None.

Poster

634. Anxiety Disorders: Preclinical Models

Location: Halls B-H

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Program#/Poster#: 634.17/GGG10

Topic: G.05. Anxiety Disorders

Title: Subchronic treatment with the immunosuppressant rapamycin alters behavior in rats

Authors: ***M. HADAMITZKY**, L. LÜCKEMANN, I. BENDIX, H. ENGLER, M. SCHEDLOWSKI;
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Abstract: The immunosuppressant rapamycin inhibits the activity of the mammalian target of rapamycin (mTOR), a protein kinase that is known to play an important role in cell growth, cell proliferation, and antibody production. Due to its antiproliferative and immunosuppressive properties this drug is widely used in clinical situations such as prevention of acute graft rejection after organ transplantation, as anti-tumor medication, or for the treatment of autoimmune diseases. In the brain mTOR-signaling appears to be important as well since it is involved in different physiological and pathophysiological processes. However, clinical observations show that patients undergoing small-molecule drug immunosuppression frequently suffer from mood and anxiety disorders. Whether and to what extent these neuropsychiatric disturbances occur as direct result of the patient's medical history or are attributed to treatment with the immunosuppressive drugs remains elusive in most cases. Based on our previous work, analyzing the effects of acute rapamycin on brain activity and behavior the present study investigated in rats whether and to what extent subchronic treatment with this mTOR inhibitor, better reflecting the clinical conditions in patients, affects behavior in rats, as well. We here show that subchronic rapamycin in therapeutically relevant doses increased anxiety-like behavior while depressive-like behavior and locomotor activity were not affected. Our results indicate that treatment with rapamycin may play a role in the development of unfavorable effects on neurobehavioral outcomes in patients undergoing immunosuppressive therapy with mTOR inhibitors.

Disclosures: M. Hadamitzky: None. **L. Lückemann:** None. **I. Bendix:** None. **H. Engler:** None. **M. Schedlowski:** None.

Poster

634. Anxiety Disorders: Preclinical Models

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Topic: G.05. Anxiety Disorders

Support: VA Merit Award BX000218

VA Merit Award CX000771

Title: Models of chronic opioid exposure on avoidance behavior

Authors: *K. D. BECK[1], S. SUBBIE[2], J. SHEYNIN[3], J. FRAGALE[2], I. SMITH[4], C. E. MYERS[5];
[1]Neurosci., East Orange VAMC/NJ Med. Sch., East Orange, NJ; [2]Rutgers - Grad. Sch. of

Biomed. Sci., Newark, NJ; [3]Ann Arbor VAHCS / Univ. Michigan Med. Sch., Ann Arbor, MI; [4]Veterans Biomed. Res. Fndn., East Orange, NJ; [5]East Orange VAMC/NJMS, East Orange, NJ

Abstract: Previous research (Sheynin et al. 2016) reported that male patients in an opioid-maintenance program (e.g. methadone maintenance) acquire active avoidance behavior quicker than females in the same program or male/females control subjects. Moreover, those male patients were slow to extinguish the avoidance behavior. This study was conducted to test whether chronic exposure to methadone facilitates acquisition of an active avoidance operant behavior (lever-press) in both male Sprague Dawley rats and in an established computational model of avoidance learning. Blood measures of plasma methadone levels were assessed as the daily dosing increased to a maximum dose of 5 mg/kg. Based on those data, rats in a subsequent experiment were randomly assigned to receive 5 mg/kg methadone (i.p.), or saline vehicle, daily throughout an avoidance learning experiment. Lever-press behavior was to be acquired in order to avoid intermittent footshock, preceded by a 60 s auditory signal. Twelve acquisition sessions occurred after the first 7 days of methadone exposure. This was followed by 6 extinction sessions where the shock was no longer present. No differences were observed in the avoidance behavior; however, lower body weight was measured in those rats treated with methadone during the avoidance training period (not prior to the avoidance training). In order to try to bridge these observations to the prior human data, a computational model of avoidance learning was employed. Parameters previously associated with specific aspects of the learning process were adjusted in the computational model in order to show how the rat model may need to be altered in order to have greater face validity to the previously published human data.

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Poster

634. Anxiety Disorders: Preclinical Models

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Support: 'Ricerca Corrente', Italian Ministry of Health (grant no: RC14C)

Ateneo 2011, Sapienza University of Rome

SIR "RBSI14G1HH", Italian Ministry of Education, Universities and Research

Title: Stress response and anxiety in mice lacking microRNA34

Authors: V. CESTARI[1], D. ANDOLINA[2], M. DI SEGNI[2], E. BISICCHIA[3], F. D'ALESSANDRO[4], A. VENTURA[5], C. P. CONCEPCION[5], S. PUGLISI-ALLEGRA[1], *R. VENTURA[6];
[1]Dept. di Psicologia - Sapienza Univ. of Rome, Roma, Italy; [2]Dept. di Psicologia - Sapienza Univ. of Rome, Rome, Italy; [3]Santa Lucia Fndn., Rome, Italy; [4]IBCN - CNR, Roma, Italy; [5]Cancer Biol. and Genet. Program, Mem. Sloan-Kettering Cancer, New York, NY; [6]Univerity la Sapienza Rome, Rome, Italy

Abstract: Interactions between genetic and environmental factors might be responsible for the onset of stress-related psychiatric disorders, including anxiety. The release of prefrontal amygdala neurotransmitters is increased by stressful experiences, a response that is relevant to cognitive, emotional, and behavioral coping. In addition, stress exposure elicits anxiety-like behavior and dendritic remodeling in the amygdala. Members of the miR-34 family have been suggested to regulate synaptic plasticity and neurotransmission processes, which mediate stress-related disorders. In the present study, we evaluated acute stress-induced basolateral amygdala (BLA)-GABAergic and medial prefrontal cortex (mpFC) aminergic outflow by intracerebral in vivo microdialysis in mice harboring targeted deletions of all 3 members of the miR-34-family (miR-34-TKO). Further, we also examined fear conditioning/extinction, stress-induced anxiety, and dendritic remodeling in the BLA of stress-exposed TKO mice. Our data show that miR-34-TKO mice are characterized by resilience to stress-induced anxiety and facilitation in fear extinction. Consequently, in TKO mice no significant increase was observed in aminergic prefrontal or amygdala GABA release and no significant acute stress-induced amygdalar dendritic remodeling was evident. Differential GRM7, 5-HT2C, and CRFR1 mRNA expression was noted in the mpFC and BLA between TKO and WT mice. Our results clearly indicate that the miR-34 plays a crucial role in regulating the behavioral and neurochemical response to acute stress and in inducing stress-related amygdala neuroplasticity.

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Poster

634. Anxiety Disorders: Preclinical Models

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Topic: G.05. Anxiety Disorders

Support: NIH Grant MH090297

Title: Broadening our understanding of intra-BLA NPY-induced stress resilience assessed with cued fear conditioning

Authors: *M. BOMPOLAKI[1], J. A. ROSENKRANZ[2], W. F. COLMERS[4], J. H. URBAN[3]; [2]Dept. of Cell. and Mol. Pharmacology, Chicago Med. Sch., [3]Physiol. and Biophysics, Chicago Med. Sch., [1]Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL; [4]Dept. of Pharmacol., Univ. of Alberta, Edmonton, AB, Canada

Abstract: The anxiolytic and stress-buffering properties of neuropeptide Y (NPY) in the basolateral amygdala (BLA) have long been established in animal models as well as humans. Acute NPY administration in the BLA is anxiolytic as assessed with the social interaction paradigm and also plays an important role in fear extinction (Sadjyk et al., 1999; 2006; Gutman et al., 2008). Repeated injections of NPY in the BLA induce a long-lasting behavioral resilience to restraint stress (Sadjyk et al., 2008). These studies were designed to examine whether a BLA-mediated behavior would be affected in the model of NPY-induced stress resilience and tested whether the expression and/or extinction of cued conditioned fear would be altered in the model of NPY-induced stress resilience. Male rats received cannulae directed to the BLA (A/P -2.7mm; M/L +/-5.0mm; D/V -7.00mm) and repeated bilateral infusions of 10pmol NPY or vehicle (100 nl; 0.1M PBS) daily for 5 days. Social interaction was assessed at d1 and d8 to verify the efficacy of the treatment. Animals receiving NPY treatment have social interaction times that were significantly elevated from vehicle control. Two weeks after the injections, the animals underwent a traditional cued fear conditioning protocol: On day 1, animals were placed in chamber 1 and received 4 pairings of tone (1500Hz, 85dB) with foot shock (0.4mAmps; fear acquisition). The following day the rats were placed in a novel chamber and received 15 trials of the tone without foot shock at 60s intertrial intervals (fear extinction). On day 3, they were placed back in chamber 2 where they underwent the same protocol of tone presentation without foot shock to test for consolidation of extinction. On all three days, freezing behavior was monitored using the Anymaze software. Both vehicle- and NPY-treated rats displayed similar rate and amplitude of fear acquisition on day 1. On day 2, NPY-treated rats expressed significantly lower freezing in the first four sessions of the extinction phase and returned faster to their baseline freezing levels. On day 3, all rats expressed very little freezing behavior suggesting that extinction was equally consolidated. These results further validate the importance of NPY playing a role in buffering the expression of stress and reinforce the argument that repeated administration of intra-BLA NPY establishes an anxiolytic-like background that is also conducive to resilience. Importantly, NPY treatment does not impair the animals' ability to react effectively to a threat, but it does improve their ability to extinguish the associated learned fear. These findings can be of great importance for the way anxiety disorders like PTSD are viewed and treated.

Disclosures: M. Bompolaki: None. **J.A. Rosenkranz:** None. **W.F. Colmers:** None. **J.H. Urban:** None.

Poster

634. Anxiety Disorders: Preclinical Models

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Support: Health Research Council of New Zealand

Brain Health Research Centre, University of Otago

Title: Goal conflict rhythmicity in a bimanual anticipatory response inhibition task shows asymmetric laterality

Authors: *S. M. SHADLI, O. HIGH, N. MCNAUGHTON;
Dept. of Psychology, Univ. of Otago, Dunedin, New Zealand

Abstract: Right frontal goal-conflict-specific-rhythmicity (GCSR) in a simple stop signal task (SST) is a potential human EEG anxiety biomarker. Anticipatory response inhibition tasks (ARITs) produce a similar stopping challenge but without the pressure of a speeded go response. With left-, right-, and both-handed stopping alternatives, the ARIT is reported by participants to generate substantial subjective anticipatory conflict. We predicted that it would show particularly large GCSR. We modified a standard ARIT to match our previous SST in having three sets of stop signal delay (SSD) values: short, intermediate, and long. The intermediate SSD values tracked 50% correct stopping to maximize goal conflict, short and long SSD values were set towards the end and start of a trial, respectively. Right frontal (F8) GCSR (the EEG power contrast of stop-go x quadratic of SSD type) was compared across stop left, stop both, and stop right trials and tested for correlations with trait anxiety and neuroticism. Stop left produced 5-12 Hz GCSR that significantly correlated with trait anxiety and neuroticism. Stop right and stop both produced only 9-10Hz GCSR, which did not correlate with trait anxiety or neuroticism. SSRT for the stop both condition was 224 ms; for stop left, 370 ms; and stop right, 570 ms. sLORETA located the overall stop-related 4-12 Hz frequency power source in the right inferior frontal gyrus (rIFG) for stop left and stop right conditions. rIFG is known to control to SST stopping. For the stop both condition, the source was the uncus. sLORETA localized the GCSR power source to rIFG for stop left and to the middle frontal gyrus for stop both, consistent with previous localization of stopping. However, power *suppression* was localized in rIFG for stop right - linked to the negative GCSR power observed in the lower frequency band (5-8 Hz). The source localization data suggests that goal conflict, like stopping, can involve more than one circuit depending on whether slower or faster go responses are being emitted, consistent with previous findings. These results show that elicitation of right frontal GCSR generalizes from the SST to the ARIT and is stronger with left hand than right hand stopping; but is not stronger in the ARIT than SST.

Disclosures: **S.M. Shadli:** A. Employment/Salary (full or part-time): Postdoctoral Fellow. 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; Health Research Council of New Zealand. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Department of Psychology, University of Otago. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); N/A. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); N/A. F. Consulting Fees (e.g., advisory boards); N/A. **O. High:** None. **N. McNaughton:** A. Employment/Salary (full or part-time): Professor. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; University of Otago. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Health Research Council of New Zealand, University of Otago, Department of Psychology. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); N/A. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); N/A. F. Consulting Fees (e.g., advisory boards); Dr McNaughton has a confidential disclosure and consultingagreement with Janssen Research & Development, LLC..

Poster

634. Anxiety Disorders: Preclinical Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 634.22/GGG15

Topic: G.05. Anxiety Disorders

Title: Social modulation of conditioned and innate fear responses

Authors: V. A. GUTZEIT, 10032[1,2], M. SADSAD[3], T. L. SANTOS[3,1], S. ONG[3], R. HEN[1,4], C. A. DENNY[4,1], *Z. R. DONALDSON[4,5,6,1];
[1]Div. of Integrative Neurosci., Res. Fndn. for Mental Hyg., New York, NY; [2]Weill Cornell Med. Sci., New York, NY; [3]Barnard Col., New York, NY; [4]Columbia Univ., New York, NY; [5]Molecular, Cellular, and Developmental Biol., [6]Psychology and Neurosci., Univ. of Colorado, Boulder, Boulder, CO

Abstract: Amongst social animals, the presence of a conspecific companion can have powerful effects on mood and behavior. In particular, the social modulation of fear and anxiety represents

a highly conserved trait, as both humans and rodents display decreases in anxiety and stress responses when an affiliative conspecific is present. However, the neural processes underlying this social buffering phenomenon remain largely unknown. Our group has found that in mice, conspecific presence decreases freezing in both conditioned and innate fearful contexts. In order to identify neural populations that may contribute to social buffering of fear responses, we used a mouse line in which *Arc*-expressing neurons are indelibly labeled following interaction with a novel conspecific. We identified a subset of cells within the infralimbic prefrontal cortex (ILPFC) of male and female ArcCreER^{T2} mice that are labeled in response to interacting with a novel, ovariectomized female conspecific but not in response to a toy mouse, novel object, or food reward. Optogenetic activation of conspecific-labeled ChR2-eYFP^{+} neurons decreases freezing in contextual and innate fearful contexts, without impacting locomotion or eliciting reward, recapitulating the effects of conspecific presence. Together, these studies suggest that activation of neurons associated with an affiliative conspecific in the ILPFC may be sufficient to mediate the effects of conspecific presence on fear responses. Thus, targeting this cell population may provide novel therapeutic opportunities that harness circuits naturally engaged by social interaction in order to treat fear and anxiety-related disorders.

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Poster

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NIMH RO1 MH064433

USC Magellan Scholar Undergraduate Research Grant

USC VP for Research ASPIRE II

Title: Individual differences in behavioral and plasma hormone responses to predator stress: Analysis of corticosterone, catecholamines, cytokines, and NPY

Authors: G. H. HARTSHORN[1], K. F. KAIGLER[1], A. C. SHARKO[1], S. K. WOOD[1], C. WOOD[1], J. NYLAND[2], J. R. FADEL[1], *M. A. WILSON[1,3];

[1]Dept Pharmacol,Physiol,Neurosci, [2]Dept Pathology, Microbiol, Immunol., Univ. South Carolina, Sch. Med., Columbia, SC; [3]Dorn VA Med. Ctr., Columbia, SC

Abstract: The aim of this project is to further the common understanding of why some individuals are more susceptible to stress-related disorders, like Post-Traumatic Stress Disorder (PTSD), than others. Individual differences in behavioral resiliency during different types of stress have been correlated with plasma cortisol, cytokines, and markers of autonomic function including neuropeptide Y (NPY) and catecholamines. In this study we used predator (ferret) odor exposure in rodents as a model of traumatic stress to examine the plasma biomarkers associated with individual differences in behavioral responses to threat. Jugular catheters were surgically implanted in male Long Evans rats and a baseline blood sample was collected prior to behavioral analysis. The rodent was exposed to a predator (ferret) odor or control (no odor) for either 15 or 60 min in a closed cylindrical testing chamber and behavioral responses (freezing, jumping, towel exploration, grooming, and rearing) were recorded. Blood samples (100 µl) were taken via the jugular catheter at 15, 30, and 60 min after predator stress along with another baseline sample taken 24-48 hours following the testing. Plasma samples were analyzed for stress hormone levels using ELISAs (NPY and corticosterone), HPLC (epinephrine, norepinephrine, and serotonin), and BioPlex analysis for cytokine levels. The recorded behavioral responses were analyzed using FreezeScan (Clever Systems Inc.) automated software as well as hand scoring, and stress hormone levels were correlated with individual differences in behavior during predator stress. Results indicate that exposure to predator odor decreased latency to freeze and towel interaction, and increased rearing behaviors, compared to the no-odor control group. Exposure to predator odor induced a sustained increase in plasma corticosterone for at least 60 min, but no significant correlations were seen between corticosterone responses and behavioral endpoints. Although exposure to predator odor failed to significantly alter plasma NPY levels, a significant correlation was seen between NPY levels at 60 min and freezing behavior. Additional analyses are comparing plasma catecholamines (norepinephrine, epinephrine) as additional biomarkers of sympathetic nervous system activation and cytokine responses following predator-induced stress. Such studies help inform our understanding of the systems underlying resiliency and susceptibility in long-term responses to traumatic stress, as well as the potential of plasma biomarkers to predict behavioral responses to stress.

Disclosures: **G.H. Hartshorn:** None. **K.F. Kaigler:** None. **A.C. Sharko:** None. **S.K. Wood:** None. **C. Wood:** None. **J. Nyland:** None. **J.R. Fadel:** None. **M.A. Wilson:** A. Employment/Salary (full or part-time): Dorn VA Medical Center.

Poster

634. Anxiety Disorders: Preclinical Models

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Topic: G.05. Anxiety Disorders

Support: CAPES

CNPq

Title: Inhibition of the nitrergic neurotransmission in the dorsal periaqueductal gray promotes panicolitic-like effect in rats exposed to hypoxia condition.

Authors: *A. SPIACCI, JR[1], G. GRIPP-FERNANDES[1], D. E. RIBEIRO[1], C. BIOJONE[2], P. C. CASAROTTO[2], H. ZANGROSSI, Jr[1];
[1]Sch. of Med. of Ribeirao Preto- USP, Ribeirao Preto, Brazil; [2]Univ. of Helsinki, Helsinki, Finland

Abstract: Objectives: It has been proposed that spontaneous panic attacks are the outcome of the misfiring of an evolved suffocation alarm system. Moreover, evidences suggests that hypoxia challenge is an effective stimulus to evoke panic attack in both humans and rats. Since nitric oxide (NO) plays an important hypoxia-signaling role, we evaluated the effects promotes by systemic injection of the nitric oxide synthase inhibitor aminoguanidine on panic-like escape response elicited in rats exposed to hypoxia condition. In addition, since the dorsal periaqueductal gray (dPAG), a mesencephalic structure associated to panic, harbors a hypoxia sensitive suffocation alarm system, we also investigated if similar responses would be achieved by intra-dorsal periaqueductal gray (dPAG) injection of drugs that impair the nitrergic neurotransmission. **Methods:** Male Wistar rats were injected (7days) with aminoguanidine (0, 15, 30 or 60 mg/Kg, i.p) and submitted to hypoxia chamber 30 min after the last injection. Into the chamber, after 5 min of nomoxic condition, nitrogen was flushed into the chamber up to the production of hypoxia (7% O2). The number jumps were computed during the hypoxia challenge. In another experiment, the animals were submitted to hypoxia 10 minutes after intra-dPAG injection of the NO scavenger Carboxy-PTIO (0, 0.3, 1 or 3 nmol) or the neuronal nitric oxide synthase inhibitor N-propyl-L-arginina (0, 0.04, 0.4 or 4 nmol). **Results:** Systemic injection of aminoguanidine (15 and 30 mg/Kg) reduce the number of jumps (mean ± SEM): [sal = 28 ± 8; AG 15 = 9 ± 1; AG 30 = 12 ± 2] suggesting a panicolytic-like effect. Similar results were observed with both intra-dPAG injection of Carboxy-PTIO [sal = 23 ± 2; C-PTIO 3 = 3 ± 1] and N-propyl-L-arginina. **Conclusion:** NO seems crucial for the expression of escape behavior evoked by hypoxia condition that has been associated with panic attack. Moreover, inhibition of dPAG nitrergic neurotransmission effectively reduces the escape behavior.

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Poster

634. Anxiety Disorders: Preclinical Models

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UW HealthEmotions Research Institute

Wisconsin National Primate Research Center

Harlow Center for Biological Psychology

Title: Characterizing neuropeptide expression in the primate central extended amygdala to provide a basis for understanding mechanisms underlying anxiety

Authors: *R. KOVNER[1], A. S. FOX[1], T. SOUAIAIA[2], P. H. ROSEBOOM[1], D. A. FRENCH[1], J. A. OLER[1], J. L. FUDGE[3], J. A. KNOWLES[2], N. H. KALIN[1];
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Abstract: The central extended amygdala (EAc) is an integral part of the neural circuit mediating fear and anxiety. Our laboratory has demonstrated that two major nodes of the EAc, the central nucleus of the amygdala (Ce) and the bed nucleus of the stria terminalis (BST) are key regions in the neural circuitry mediating non-human primate (NHP) anxious temperament (AT). It is important to characterize EAc cell types in NHPs to better understand the cellular and molecular alterations that mediate AT. The Ce and BST are primarily composed of striatal-like medium spiny GABAergic neurons that can be subdivided into multiple types based on their neuropeptide profile. Although the comparison between striatal neurons and EAc neurons has been made based on shape and GABA expression, the molecular differences between the neurons of these regions have not been investigated. Using human microarray data from the Allen Brain Atlas, we identified genes that are differentially expressed between Ce and striatum. We further validated this finding in the NHP using RNA sequencing and found that many neuropeptides have significantly higher expression in the lateral Ce (CeL) compared to regions of striatum (Putamen and Caudate). Using this human and NHP gene data as a guide, we chose to study somatostatin (SST) and cholecystokinin (CCK) in more depth in the NHP EAc. To this

end, we used *in situ* hybridization to characterize the anterior-posterior (A-P) distribution of SST and CCK mRNA expression. A-P distribution is interesting because rodent work has demonstrated differences in peptide expression between anterior and posterior regions of the CeL. However, little work has been done in the NHP. To start, we focused on the expression of the SST and CCK mRNA in CeL and the corresponding laterodorsal BST (BSTL) from 4 rhesus monkeys. Using a repeated-measures multiple regression analysis we examined the effect of A-P position on expression levels. Results demonstrated a significant A-P effect on CeL-SST expression, such that SST expression increases towards the posterior CeL ($F_{4, 30}$=19.29,p=0.02). There was no significant effect of A-P position on BSTL-SST expression. We did not observe any significant effects of A-P position on CCK expression in CeL or BSTL. These experiments utilize gene data to identify differentially expressed mRNAs between striatum and Ce as a stepping-stone to identify differences in the A-P expression of neuropeptides within the primate CeL and BSTL. Understanding the A-P expression of different neuropeptide populations in the EAc will help to guide co-expression studies, to identify different neural populations and determine population-specific roles in mediating AT.

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Poster

634. Anxiety Disorders: Preclinical Models

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

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Topic: G.05. Anxiety Disorders

Support: NIH Grant MH081884

Title: Effects of caudal orbitofrontal cortex 'strip lesions' on threat-related behavior and brain metabolism in young female rhesus monkeys

Authors: *J. A. OLER[1], A. S. FOX[2], D. P. M. TROMP[1], M. K. RIEDEL[1], E. M. FEKETE[1], P. H. ROSEBOOM[1], E. A. MURRAY[3], N. H. KALIN[1];
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Abstract: Persistent and high levels of sustained anxiety, or an anxious temperament (AT), during childhood is one of the strongest risk factors for the later development of stress-related psychopathology. The nonhuman primate model of AT is ideal because of similarities between

rhesus monkeys and humans in the development of socio-emotional behavior and its underlying neural circuits, which are characterized by a high degree of integration between subcortical structures and the primates' well-developed prefrontal cortex. The orbital prefrontal cortex (OFC) is thought to play a critical role in the regulation of emotions, which is consistent with our demonstration of increased AT-related metabolism, and a significant genetic correlation between AT and metabolism within caudal OFC regions (Fox et al., 2015). Moreover, lesions of the primate OFC reduce threat-related behavioral responses (Kalin et al., 2007; Murray & Izquierdo, 2007; Bachevalier et al., 2011) and attenuate glucose metabolism within the extended amygdala (Fox et al., 2010). As part of an ongoing study examining the role of the OFC in regulating the extended amygdala at functional and molecular levels, we performed aspiration lesions of a narrow strip of the caudal OFC in six female rhesus monkeys (mean age = 2.18 +/- 0.19 years, including cage-mate controls). These OFC "strip lesions" have been shown to attenuate responses to threatening stimuli (e.g., snakes; Rudebeck et al., 2013). Structural brain imaging using diffusion weighted tractography methods demonstrate the disconnecting effects of the strip lesion on the white matter coursing through the caudal OFC. In behavioral assessments using the Human Intruder Paradigm, monkeys with caudal OFC strip lesions exhibited significant decreases in threat-related responding during the No-Eye-Contact condition (Post - Pre Δ in AT, $p < 0.05$). Preliminary FDG-PET imaging results suggest a replication of previously published findings in monkeys with large OFC lesions. Specifically, threat-induced glucose metabolism in the bed nucleus of the stria terminalis (a major component of the extended amygdala) is diminished following OFC strip lesions ($p < 0.005$, uncorrected). These results demonstrate that the gray matter and/or the white matter tracts running along/through the caudal OFC (e.g., uncinate fasciculus) are critical for the processing of threat-related information and the expression of adaptive behavioral responses to threat. All procedures were performed according to federal and institutional guidelines at the Harlow Center for Biological Psychology and the Wisconsin National Primate Research Center.

Disclosures: **J.A. Oler:** None. **A.S. Fox:** None. **D.P.M. Tromp:** None. **M.K. Riedel:** None. **E.M. Fekete:** None. **P.H. Roseboom:** None. **E.A. Murray:** None. **N.H. Kalin:** None.

Poster

634. Anxiety Disorders: Preclinical Models

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UW HealthEmotions Research Institute

Wisconsin National Primate Research Center

Title: Use of designer receptors exclusively activated by designer drugs (DREADDs) to investigate the molecular underpinnings of anxious temperament in non-human primates.

Authors: *P. H. ROSEBOOM[1], A. S. FOX[1], J. A. OLER[1], M. E. OLSEN[2], E. K. BRODSKY[2], R. KOVNER[1], M. K. RIEDEL[1], E. M. FEKETE[1], Y. XIONG[4], W. F. BLOCK[2], A. L. ALEXANDER[3], R. M. BIRN[3], J. JIN[4], N. H. KALIN[1];
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Abstract: Extreme anxiety in children is a prominent predictor of the later development of psychopathologies including anxiety disorders and depression. Using our well-established non-human primate (NHP) model of anxious temperament (AT), we identified the central nucleus of the amygdala (Ce) as a key component of the neural circuit that underlies the expression of AT. Using positron emission tomography, we found that Ce metabolic activity strongly predicts individual differences in AT, and that selective Ce excitotoxic lesions decrease AT. To further define the role of the Ce, we have begun to employ Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to selectively modulate neuronal activity within the amygdala in response to the administration of the DREADDs receptor ligands clozapine-N-oxide (CNO) or the related analog compound 21 (C21). Because CNO and C21 are known to have some off target effects in NHPs, we first assessed their effects on anxiety-related behavior in the no eye contact (NEC) condition of the human intruder paradigm. Systemic administration to rhesus monkeys (*Macaca mulatta*) of either CNO (0, 5 or 10 mg/kg; N=5/group) or C21 (0, 3 or 9 mg/kg; N=5/group) had no significant effects on NEC-induced freezing (CNO, $F_{2,12}$=2.18; p=0.16; C21, $F_{2,12}$=1.37; p=0.29) or plasma cortisol (CNO, $F_{2,12}$=2.79; p=0.10; C21, $F_{2,12}$=0.78; p=0.48). We then moved on to localized delivery of an adeno-associated viral vector type 5 that directs expression of the DREADDs version of the inhibitory human M4 muscarinic receptor as well as mCherry protein under the control of the synapsin 1 promoter (AAV5/hSyn-hM4Di-mCherry). Using real time magnetic resonance imaging (MRI)-guided surgical techniques in a cynomolgus monkey (*Macaca fascicularis*), the AAV5 viral construct was infused bilaterally targeting the Ce. Seven weeks later the monkey was sacrificed and the brain was processed for immunohistochemistry. Consistent with the extent of the gadolinium cloud visible during the MRI-guided infusion, mCherry protein was expressed in both hemispheres in regions extending beyond but encompassing the Ce. In the next study, 5 rhesus monkeys received smaller infusions

of the same viral vector into the Ce. Several months after the viral infusion, administration of either CNO (5 mg/kg) or C21 (9 mg/kg) consistently decreased NEC-induced freezing compared to vehicle in three of the five animals tested. Ongoing metabolic and functional connectivity imaging studies are identifying CNO-induced alterations. The current study shows the feasibility of using DREADDs technology in NHPs to study neural circuits underlying the expression of anxiety-related behavior.

Disclosures: **P.H. Roseboom:** None. **A.S. Fox:** None. **J.A. Oler:** None. **M.E. Olsen:** None. **E.K. Brodsky:** None. **R. Kovner:** None. **M.K. Riedel:** None. **E.M. Fekete:** None. **Y. Xiong:** None. **W.F. Block:** None. **A.L. Alexander:** None. **R.M. Birn:** None. **J. Jin:** None. **N.H. Kalin:** None.

Poster

635. Anxiety Disorders: Therapeutic Approaches

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 635.01/GGG21

Topic: G.05. Anxiety Disorders

Title: MDMA-assisted psychotherapy for treatment of chronic post traumatic stress disorder: findings from MAPS-sponsored phase 2 clinical research trials

Authors: *A. A. FEDUCCIA[1], B. YAZAR-KLOSINSKI[2], L. JEROME[1], M. MITHOEFER[1], A. EMERSON[1], R. DOBLIN[2];
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Abstract: The Multidisciplinary Association for Psychedelic Studies (MAPS) has completed six sponsored Phase 2 clinical pilot studies that investigated MDMA-assisted psychotherapy for treating chronic, treatment-refractory posttraumatic stress disorder (PTSD). At five study sites world-wide, 108 subjects underwent a series of non-drug preparatory and integration sessions, interspersed with two or three double-blind, MDMA-assisted psychotherapy sessions with a comparator/placebo control. Intent-to-treat findings from these studies showed medium to large between-subjects effect size in reducing PTSD symptoms after two sessions, as measured by the Clinician Administered PTSD Scale (CAPS-4), with a good safety profile. These positive results provide a basis for the continuation of the MAPS-sponsored research program into expanded Phase 3 clinical trials to provide safety and efficacy data for use of MDMA as a pharmacological adjunct to therapy for treatment of PTSD. Given the limited effectiveness of current available medications and therapeutic strategies in some people, MDMA-assisted psychotherapy holds promise as a novel treatment option and warrants further study.

Disclosures: **A.A. Feduccia:** None. **B. Yazar-Klosinski:** None. **L. Jerome:** None. **M. Mithoefer:** 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; Contracted research. **A. Emerson:** None. **R. Doblin:** None.

Poster

635. Anxiety Disorders: Therapeutic Approaches

Location: Halls B-H

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Program#/Poster#: 635.02/GGG22

Topic: G.05. Anxiety Disorders

Title: Safety of mdma therapy for social anxiety in autistic adults

Authors: *B. B. YAZAR-KLOSINSKI[1], C. S. GROB[2], A. DANFORTH[2];
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Abstract: Autistic adults who are verbal and whose autism might not be immediately recognizable to others often initially present in a clinical setting with a co-morbid diagnosis of anxiety or depression, and are at greater risk of experiencing social anxiety due to core features of autism. There are currently no FDA-approved pharmacologic treatments for autistic adults, although off-label prescription use of selective serotonin reuptake inhibitors (SSRIs) are on the rise in this population. The Multidisciplinary Association for Psychedelic Studies (MAPS) is sponsoring an exploratory Phase 2 dose-finding safety and feasibility pilot study investigating MDMA-assisted therapy for treating social anxiety in autistic adults. After three non-drug preparatory therapy sessions, 12 subjects received two double-blind MDMA-assisted therapy sessions with a placebo control, each followed by three integrative therapy visits. Participants were followed monthly during a blinded 6-month follow-up. A blinded clinician administered the Leibowitz Social Anxiety Scale (LSAS) and found promising rapid-onset reduction in social anxiety symptoms in the MDMA group with a good safety profile in preliminary analysis. Changes in social perception, emotion labeling and regulation, perceived stress, self-esteem, state and trait anxiety, depression, and quality of life were also assessed as secondary outcomes. The promising results from this small sample will be used to estimate statistical power for future studies of this novel treatment option.

Disclosures: **B.B. Yazar-Klosinski:** A. Employment/Salary (full or part-time): MAPS. **C.S. Grob:** 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; Los Angeles Biomedical

Research Institute, MAPS. **A. Danforth:** 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; Los Angles Biomedical Research Institute, MAPS.

Poster

635. Anxiety Disorders: Therapeutic Approaches

Location: Halls B-H

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Topic: G.05. Anxiety Disorders

Support: NIMH R01MH087826

Title: Identification of the first variant selective and biased NPS Receptor agonist that retains anxiolytic and memory promoting effects with reduced levels of locomotor stimulation

Authors: *S. CLARK[1], C. HASSLER[2], S. GERTZ[1], E. GAY[2], S. RUNYON[2];
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Abstract: Neuropeptide S is a 20-amino acid peptide that functions as an agonist through activation of its cognate GPCR receptor system. High expression of the Neuropeptide S (NPS) receptor has been found in the retrosplenial cortex, basolateral amygdala, parasubiculum, and various regions of the hypothalamus. Modulation of the NPS receptor has been associated with a variety of disease states including anxiety, panic disorder, PTSD, and substance abuse. Through comprehensive chemical modification and analog synthesis we have now defined a comprehensive NPS agonist pharmacophore using traditional medicinal chemistry and computational/scaffold hopping approaches. The compounds developed in our laboratory selectively activate calcium mobilization in vitro. In mice, these compounds selectively modulate a subset of behaviors for which full NPS receptor agonists are known to elicit. Further modification and testing of these analogs will be of significant value in determining the comprehensive role of NPS in modulating anxiety and responses to stress.

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Poster

635. Anxiety Disorders: Therapeutic Approaches

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Topic: G.05. Anxiety Disorders

Support: Ministry of Science and Technology of China 2013CB835100

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National Natural Science Foundation of China 81401107

Title: κ Opioid receptor activation in different brain regions differentially modulates anxiety-related behaviors in mice

Authors: *Y. WANG, A. HANG, J.-G. LIU;
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Abstract: κ Opioid receptor system is widely implicated in the regulation of emotion. However, the findings about the role on anxiety in rodents are highly controversial, since both anxiogenic- and anxiolytic-like effects have been reported with κ opioid receptor activation. The mechanism and the underlying neuroanatomical substrates are unexplored. In the present study, we first investigated the effects of κ agonist U50,488H on anxiety-related behaviors over a wide range of doses, and we found that U50,488H produced dual effects in anxiety, with low dose being anxiogenic and high dose being anxiolytic. To assess the potential neuroanatomical substrates, we used phosphorylation of extracellular signal-related kinase1/2 (pERK1/2) to map the underlying neural circuits. We found that the anxiogenic effect of U50,488H was paralleled by an increase of pERK1/2 in the nucleus accumbens, whereas the anxiolytic effect was paralleled by an increase of pERK1/2 in the lateral septal nucleus. We then examined the behavioral consequences with locally microinjection of U50,488H, and we found that microinjection of U50,488H into the nucleus accumbens exerted anxiogenic-like effects, whereas microinjection of U50,488H into the lateral septal nucleus. Both effects can be abolished by κ antagonist nor-BNI pretreatment. To the best of our knowledge, the present work firstly provides the neuroanatomical sites that mediating the dual anxiogenic- and anxiolytic-like effects of U50,488H in mice. This study may help to explain current controversial role of κ receptor activation in anxiety-related behaviors in rodents, and may open new perspectives in the areas of anxiety disorders and κ receptor function.

Disclosures: **Y. Wang:** None. **A. Hang:** None. **J. Liu:** None.

Poster

635. Anxiety Disorders: Therapeutic Approaches

Location: Halls B-H

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Program#/Poster#: 635.05/GGG25

Topic: G.05. Anxiety Disorders

Support: DFG ZL 59/2-1

DFG ZL 59/2-2

Title: The effects of post-exposure gluccocorticoid administration in specific phobia

Authors: *A. ZLOMUZICA[1], F. PREUSSER[1], C. MERZ[2], O. T. WOLF[2], M. TEGENTHOFF[3], J. MARGRAF[1];
[2]Dept. of Cognitive Psychology, [1]Ruhr-Universität Bochum, Bochum, Germany; [3]BG-Kliniken Bergmannsheil, Bochum, Germany

Abstract: Glucocorticoids are important modulators of emotional learning and memory. Glucocorticoid administration prior to therapy has been shown to enhance the efficacy of exposure-based treatments. However, the exact mechanisms of action remain poorly understood: Glucocorticoids may exert their beneficial effects through a) inhibiting fear memory retrieval, or b) promoting the formation and consolidation of corrective experiences. Here, by administering glucocorticoids after exposure therapy, we tested whether the enhancing effects of glucocorticoids on therapeutic outcome can be attributed to extinction memory consolidation and whether glucocorticoids do also attenuate fear renewal. Compared to placebo, post-exposure glucocorticoid administration did neither lead to more anxiety reduction nor to less fear renewal at both the behavioral and subjective level. Rather than enhancing the consolidation of extinction memories, our preliminary findings support the view that glucocorticoids might exert their beneficial effects on exposure therapy by promoting the formation of new corrective experiences or inhibiting aversive memory retrieval during exposure.

Disclosures: A. Zlomuzica: None. **F. Preusser:** None. **C. Merz:** None. **O.T. Wolf:** None. **M. Tegenthoff:** None. **J. Margraf:** None.

Poster

635. Anxiety Disorders: Therapeutic Approaches

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Program#/Poster#: 635.06/GGG26

Topic: G.05. Anxiety Disorders

Support: German research Foundation Grant SFB TRR 58, Project C06 and B01

Title: Medial prefrontal cortex stimulation accelerates therapy response of exposure therapy in acrophobia

Authors: *M. HERRMANN[1,2], A. KATZORKE[2], Y. BUSCH[2], D. GROMER[3], T. POLAK[2], P. PAULI[3], J. DECKERT[2];
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Abstract: Animal studies have shown that stimulation of the medial prefrontal cortex (mPFC) improves extinction of conditioned fear. This is thought to depend on an enhanced efficiency in the interplay between the mPFC and the amygdala. A recent study confirmed this in humans by showing that repetitive transcranial magnet stimulation (rTMS) of the mPFC facilitates extinction learning as evidenced in reduced responses to a conditioned threat stimulus assessed with fear potentiated startle (FPS), skin conductance response (SCR) and arousal ratings. As a crucial next translational step this study investigates whether mPFC stimulation using rTMS is an effective add-on for exposure therapy in anxiety disorder patients. Patients with acrophobia (N=43) were randomly allocated to active or sham rTMS covering the mPFC before two exposure sessions in virtual-reality (VR). Corroborating experimental animal and human studies we provide first clinical evidence that high-frequency rTMS improves exposure therapy response of acrophobia symptoms one week after the second exposure session. A follow-up three months later showed a further improvement of the acrophobia symptoms in both groups to an equal level. This study provides first evidence that stimulation of the mPFC accelerates the therapeutic effect of exposure based therapy.

Disclosures: M. Herrmann: None. **A. Katzorke:** None. **Y. Busch:** None. **D. Gromer:** None. **T. Polak:** None. **P. Pauli:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); VTPlus. **J. Deckert:** None.

Poster

635. Anxiety Disorders: Therapeutic Approaches

Location: Halls B-H

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Program#/Poster#: 635.07/HHH1

Topic: G.05. Anxiety Disorders

Title: Environmental enrichment may produce central effects in modulating the impact of high-fat diet induced anxiety in a rat model of obesity

Authors: *E. P. WIERTELAK, S. FERGUS, J. E. MEYERS-MANOR;
Neurosci. Studies, Macalester Col., Saint Paul, MN

Abstract: The increasing prevalence of obesity in Western cultures constitutes a serious public health issue. In addition to its negative physiological health outcomes, consumption of a high-fat diet (HFD) is also linked to a variety of affective disorders, including anxiety. HFD has been shown to impact a number of neurobiological functions, including alterations in hypothalamic-pituitary-adrenal (HPA) axis activity, and decreased hippocampal neurogenesis, both of which have also been implicated in the regulation of affective states. The importance of rearing environment in the regulation of brain, behavior, and physiology has also long been recognized in biological, and social sciences. Although the effects on emotionality are less documented, environmental enrichment (EE) has beneficial effects in reducing anxiety-like behavior in animals. Exposure to complex environments alters brain structure and function, through positively regulating HPA axis activity, and inducing forms of brain plasticity, such as increased hippocampal neurogenesis and BDNF levels. However, few studies have examined the interaction between the two variables. The present study established an anxiety model in Sprague-Dawley rats through a high-fat diet (HFD) to examine the effect of environmental enrichment on HFD-induced anxiety. Subjects were grouped into either a control diet or HFD group, each of which were separated into either a non-enriched or EE housing condition for 4 weeks. The balanced design of two dietary conditions by two housing conditions allowed for investigation of main effects of diet, environment, and any interactions. Anxiety was assessed using the elevated zero maze. Results indicate that HFD induced greater anxiety, as revealed by a lower percentage of time spent in the open quadrants of the maze. However, results also showed an amelioration of anxiety-like behavior if subjects were exposed to an enriched environment, despite continuation of HFD. Considering that the beneficial effect of EE was obtained even with continuation of HFD, this study provides solid evidence that EE is an effective and practical intervention to ameliorate anxiety-like behavioral changes induced from HFD. As this effect could result through targeting a number of underlying neurobiological mechanisms, future work in this laboratory is focused on these examinations.

Disclosures: **E.P. Wiertelak:** None. **S. Fergus:** None. **J.E. Meyers-Manor:** None.

Poster

635. Anxiety Disorders: Therapeutic Approaches

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Topic: G.05. Anxiety Disorders

Support: Ontario Mental Health

Title: Investigating the effects of ghrp6 in combination with citalopram to counter the metabolic and behavioral effects of chronic social defeat

Authors: *L. M. HYLAND[1], S. PARK[2], S. OOSTLANDER[1], E. VINNINS[1], A. ABIZAID, K1S5B6[1];
[2]Neurosci., [1]Carleton Univ., Ottawa, ON, Canada

Abstract: Ghrelin, a gut-derived peptide hormone, is associated with feeding, energy balance, and the stress response. Our primary goal was to test whether chronic systemic ghrelin agonist (GHRP6) administration alone, or in combination with a commonly prescribed selective serotonin reuptake inhibitor (SSRI, i.e. citalopram) would enhance antidepressant effects in mice under normal conditions or following chronic social defeat paradigm (CSDP). Interestingly, while GHRP6 treatment alone tended to increase weight in non-stressed mice, the same treatment prevented citalopram induced weight gain in stressed mice. Depression is associated with anhedonia, social deficits, and anxiety, and can be modelled in animals using tests such as the sucrose preference test, social interaction test, and the open field test. Our results indicated that after a recovery period of no stress following CSDP, there were no major effects of either GHRP6 or citalopram in improving depressive-like behaviors in these behavioral measures. These data indicates that GHRP6 may lessen the degree of metabolic side-effects seen in patients treated with SSRIs, but does not improve the behavioral effects of stress after a long recovery period.

Disclosures: L.M. Hyland: None. **S. Park:** None. **S. Oostlander:** None. **E. Vinnins:** None. **A. Abizaid:** None.

Poster

635. Anxiety Disorders: Therapeutic Approaches

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Program#/Poster#: 635.09/HHH3

Topic: G.05. Anxiety Disorders

Support: Longwood University Faculty Development Grant

Longwood University PRISM Grant

Title: Effects of wilderness therapy on neuroendocrine measures of anxiety

Authors: A. K. EAGLE[1], S. K. YEATTS[2], E. J. TEDDER[2], K. D. MCCLUSTER[2], L. MANSER[2], *C. L. FRANSSEN[2];
[1]Biol., [2]Psychology, Longwood Univ., Farmville, VA

Abstract: Conventional treatments for anxiety include pharmacotherapy and psychotherapy techniques, however no consistently effective treatment has been found. A variety of alternative therapies- many utilizing exercise and nature exposure- have emerged; however, evidence for efficacy and efficiency relies heavily on self-reported measures of affect. Wilderness therapy is uniquely positioned to incorporate elements of both nature and exercise as well as interactions with a licensed clinical practitioner; and this combinatorial approach has proven beneficial to substance abuse, behavior disorders, and mood disorders. Anxiety disorders are comorbid for many patients seeking treatment through wilderness therapy. To better understand changes in anxiety profiles through treatment, we studied patients' neuroendocrine profiles during a wilderness therapy experience. In this project, we partnered with Blackwater Outdoor Experiences, a wilderness therapy group based in Midlothian, VA. We collected saliva samples and survey data before, during, and after a 22-day wilderness therapy trip. Samples were assayed for stress-related hormones, namely cortisol and DHEA. We present our integrative data analysis here.

Disclosures: A.K. Eagle: None. **S.K. Yeatts:** None. **E.J. Tedder:** None. **K.D. McCluster:** None. **L. Manser:** None. **C.L. Franssen:** None.

Poster

635. Anxiety Disorders: Therapeutic Approaches

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Program#/Poster#: 635.10/HHH4

Topic: G.05. Anxiety Disorders

Title: Anxiolytic and antidepressant-like properties of ONO-9270578, a novel TRPC4/5 inhibitor, in mice

Authors: *T. NIWA[1], A. KISHI[1], T. KITAJIMA[1], Y. KAMIMURA[2], T. SASAMURA[2], Y. HIROKA[3], T. SAITO[3], S. KATSUMATA[1];
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Abstract: *Purpose:* Transient receptor potential canonical (TRPC) channel subfamily is highly expressed in the central nervous system. Ca^{2+} signaling through these channels that are activated following stimulation of Gq-coupled receptors and Ca^{2+} store depletion relates to various neuronal functions such as neuronal development, proliferation, differentiation and synaptogenesis. The mice lacking TRPC4 exhibited decreased anxiety-like behaviors in elevated plus maze (EPM) and open field test (OF) but not in acoustic startle response (ASR) and fear conditioning test (FCT). Also, the mice lacking TRPC5 exhibited decreased anxiety-like behaviors in EPM, OF and social interaction (SI) but not in ASR, FCT and novelty suppressed feeding test. These findings suggest that TRPC4 and TRPC5 may both relate to the mechanisms underlying regulation of anxiety and fear responses. Recently, we identified ONO-9270578 as a TRPC4/5 inhibitor and evaluated the effects of this compound in various behavioral tests. *Methods:* ONO-9270578 was examined on EPM, SI and marble burying test (MBT) in normal mice to evaluate the anxiolytic-like properties of TRPC4/5 inhibitor. The effects of ONO-9270578 were compared with diazepam (3 mg/kg), tandospirone (20 mg/kg) and zimelidine (10 mg/kg) in EPM, SI and MBT, respectively. ONO-9270578 was also examined on EPM in cholecystokinin (CCK) 2 receptor agonist CCK4-treated mice and SI in 5-HT_{2C} receptor agonist MK212-treated mice to confirm the involvement of Gq-coupled receptors in the effects of TRPC4/5 inhibitor. Additionally, ONO-9270578 was examined on OF and passive-avoidance test (PA) in olfactory bulbectomized (OB) mice to evaluate the effects of TRPC4/5 inhibitor on OB-induced hyperactivity and cognitive impairment. *Results:* ONO-9270578 significantly increased the time spent on open arms in EPM and the social interaction time in SI in normal mice. In these tests, the anxiolytic-like effects of ONO-9270578 were equivalent to comparative drugs. On the other hands, ONO-9270578 did not influence the number of buried marbles in MB. ONO-9270578 also significantly suppressed the decrease of time spent on open arms by CCK4 and that of social interaction time by MK212. Furthermore, ONO-9270578 significantly suppressed the increase of total movement distance on open-field and that of the number of trials

to learn passive avoidance in OB mice without affecting in sham-operated mice. *Conclusion:* ONO-9270578 showed anxiolytic and antidepressant-like effects on various behavioral tests in normal or pathological conditions, indicating TRPC4/5 inhibitor may have a potential as a treatment for anxiety and depression.

Disclosures: **T. Niwa:** None. **A. Kishi:** None. **T. Kitajima:** None. **Y. Kamimura:** None. **T. Sasamura:** None. **Y. Hiroka:** None. **T. Saito:** None. **S. Katsumata:** None.

Poster

635. Anxiety Disorders: Therapeutic Approaches

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 635.11/HHH5

Topic: G.05. Anxiety Disorders

Title: The effects of sceletium tortuosum extract fraction in chick anxiety-depression model.

Authors: *M. K. JOURDAN[1], J. M. CARPENTER[2], E. M. FOUNTAIN[1], Z. ALI[2], N. ABE[2], I. A. KHAN[2], K. J. SUFKA[1];
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Abstract: *Sceletium tortuosum* has been reported to elevate depressed mood, relieve pain, and reduce anxiety, stress, and tension. *S. tortuosum's* major constituents are mesembrine, mesembrone and mesembranol, among others. This study sought to examine the effects of an *S. tortuosum* alkaloid enriched fraction in the chick anxiety-depression model. This model shows high predictive validity as a pharmacological screening assay. Two experiments were conducted using socially-raised male Silver Laced Wyandotte chicks (Ideal Poultry, Cameron, TX, USA). In two experiments, separate groups of 4-6 day old chicks were given IP vehicle, Imipramine (10 mg/kg), or *S. tortuosum* fraction (10, 20, 30 mg/kg in Exp. 1 or 50, 75, 100 mg/kg in Exp. 2) 15 min prior to a 60 m isolation test period. Vehicle chicks displayed high distress vocalization (DVoc) rates in the anxiety phase (first 5 m). DVoc rates declined about 50% (i.e., behavioral despair) in the depression phase (30-60 m). Imipramine attenuated behavioral despair. None of the *S. tortuosum* fraction doses attenuated behavioral despair. However, 75 and 100 mg/kg *S. tortuosum* fraction decreased DVoc rates during the anxiety phase. The findings that an alkaloid enriched *S. tortuosum* fraction shows anxiolytic effects fits well with the observation that its major constituent mesembrine binds to GABA and mu-opioid receptors. While mesembrine also inhibits serotonin reuptake, our data show that this enriched fraction does not have antidepressant properties at the doses tested.

Disclosures: **M.K. Jourdan:** None. **J.M. Carpenter:** None. **E.M. Fountain:** None. **Z. Ali:** None. **N. Abe:** None. **I.A. Khan:** None. **K.J. Sufka:** None.

Poster

635. Anxiety Disorders: Therapeutic Approaches

Location: Halls B-H

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Topic: G.05. Anxiety Disorders

Support: NIH R01DK092587

NIH P20GM103629

NIH P30DK072476

NIH T32-DK064584

Title: Activation of lateral hypothalamic galanin neurons reduces anxiety-like behavior

Authors: ***E. QUALLS-CREEKMORE**[1,2], A. BRUCE-KELLER[2], M. FRANCOIS[2], S. YU[2], C. MORRISON[2], H. MÜNZBERG[2];
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Abstract: Pharmacological anxiolytics are one of the most widely prescribed treatments in psychiatric disease; however, the currently available pharmacological options are often associated with negative side effects including drowsiness and physical dependence. Despite the increasing prevalence of anxiety disorders, the neural circuits underlying anxiety are not clear. There are many gaps in defining the neural circuits, and in particular, the molecular identity of proposed nuclei in the anxiety circuit. Recently, the lateral hypothalamus (LHA) has been implicated in the neural circuits regulating anxiety. The LHA contains many galanin expressing neurons and galanin signaling has been strongly associated with reduced anxiety. We hypothesize that LHA galanin neurons mediate anxiety-related behavior. To test this we activated LHA galanin neurons chemogenetically. We injected a cre-dependent adenoassociated virus to drive expression of hM3-Gq receptor in the LHA of galanin-cre mice. Clozapine-N-oxide (CNO) induced activation of LHA galanin neurons was tested for anxiety related behavior in the open field test, elevated plus maze, and marble burying test. CNO significantly decreases anxiety-like behavior in all tested paradigms. Importantly, anxiolytic effects were not accompanied by reduced arousal indicated by a modest increase in locomotor activity in the open field test. This data supports further investigation of galanin as a potential pharmacological target for the treatment of anxiety without dampening arousal, which is often associated with common

anxiolytic drugs. *Supported by R01DK092587, P20GM103629 (HM) P30DK072476 (SY, HM), T32-DK064584 (EQC)*

Disclosures: **E. Qualls-Creekmore:** None. **A. Bruce-Keller:** None. **M. Francois:** None. **S. Yu:** None. **C. Morrison:** None. **H. Münzberg:** None.

Poster

635. Anxiety Disorders: Therapeutic Approaches

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Program#/Poster#: 635.13/HHH7

Topic: G.05. Anxiety Disorders

Support: NMU Foundation

Title: The effects of neurotensin receptor agonists on fear-potentiated startle in male and female mice

Authors: M. VANDEN AVOND, E. RIDGE, A. FULSCHER, *A. PRUS;
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Abstract: Neurotensin is a neuropeptide neurotransmitter with receptors located in areas of relevance to anxiety, including hippocampus and amygdala and interacts with a variety of neurotransmitter systems, including monoamines, acetylcholine, glutamate, and GABA. Central administration of neurotensin and systemic administration of brain penetrant neurotensin NTS_1 and NTS_2 receptor agonists produce anxiolytic effects, and $NTS_2^{-/-}$ mice exhibit deficits in fear conditioning. The present study sought to further evaluate the potential anxiolytic effects of a systemically-administered NTS_1 receptor agonist, PD149163, and a NTS_2 receptor agonist, beta-lactotensin, using the fear-potentiated startle (FPS) paradigm in male and female C57BL/6 mice. Startle chambers were equipped with a shock-grid floor, fluorescent light, and an acoustic startle speaker. Conditioning took place between the light and floor shock, and test sessions measured startle to a 90 dB noise burst while the light was on or off. Startle magnitude did not differ between the male and female mice. Mice were subsequently separated into two groups (termed "high responders" and "low responders") based upon their degree of FPS response produced following saline administration. PD149163 significantly reduced FPS and startle magnitude in both male and female high responders, but not in low responders. Beta-lactotensin reduced FPS in female high responders at a single dose, but did not reduce FPS in either group of male mice. The anxiolytic and partial 5-HT_{1A} agonist buspirone did not significantly reduce FPS in either male or female mice. PD149163 and buspirone, but not beta-lactotensin, significantly reduced

locomotor activity in an open field. Based on these findings, neurotensin receptors may be a novel target for anxiolytic compounds.

Disclosures: **M. Vanden Avond:** None. **E. Ridge:** None. **A. Fulscher:** None. **A. Prus:** None.

Poster

635. Anxiety Disorders: Therapeutic Approaches

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Topic: G.05. Anxiety Disorders

Support: NIH Grant MH100096

Title: Endocannabinoid modulation of reciprocal prelimbic prefrontal cortex circuits

Authors: ***D. MARCUS**, S. PATEL;
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Abstract: The prelimbic prefrontal cortex (plPFC) is a subregion of the medial prefrontal cortex (mPFC) that is importantly involved in anxiety and stress responses. Research has indicated that the activity of the mPFC and its ability to modulate stress response is heavily influenced by local endocannabinoid signaling. Local pharmacological activation of cannabinoid signaling in the mPFC induces an anxiolytic effect, while local inhibition of cannabinoid signaling prolongs corticosterone (CORT) secretion in response to acute stress and promotes an anxiogenic phenotype. Due to the primarily presynaptic localization of the cannabinoid receptor type 1 (CB1), the endocannabinoid system (ECS) may play a crucial role in gating excitatory glutamatergic inputs into the mPFC. In this study, we combine optogenetic projection targeting and retrograde labeling (retrobead) approaches to isolate reciprocal projections between the plPFC and two glutamatergic projection regions, the Basolateral Amygdala (BLA) and Mediodorsal Thalamus (MDT). Both of these regions have been implicated in playing a causal role in anxiety and stress response. Neither retrobead positive nor retrobead negative cells in plPFC layer 6 receiving projections from the DMT express depolarization induced suppression of excitation (DSE), an endocannabinoid mediated form of transient synaptic depression. However, both retrobead positive and negative cells in layer 2 receiving inputs from the BLA exhibited DSE. Finally, only layer 5 retrobead positive cells receiving input from the BLA exhibited DSE. These distinct modes of endocannabinoid signaling may underlie the behavioral effects of both local mPFC and global cannabinoid agonist administration.

Disclosures: **D. Marcus:** None. **S. Patel:** None.

Poster

636. Molecular Mechanisms of Cocaine Addiction

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 636.01/HHH9

Topic: G.08. Drugs of Abuse and Addiction

Title: Electrochemical measurements of real-time opioid peptide fluctuations reveal a neuromodulatory role for putative methionine enkephalin dynamics in the rat striatum

Authors: *C. A. LEE, S. E. CALHOUN, S. K. SMITH, C. MEUNIER, M. DAUSH, G. S. MCCARTY, L. A. SOMBERS;
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Abstract: Neuropeptides regulate a broad spectrum of biological functions, from pain and analgesia to hedonic and motivational behaviors associated with reward and addiction. Opioid signaling within dopaminergic circuits has been critically implicated in both natural and drug reward-seeking behavior. However, the precise mechanisms that underlie opioid modulation of DA systems remain ambiguous. Although several methods exist for monitoring DA fluctuations, few tools are available for selectively monitoring dynamic fluctuations of endogenous opioid neuropeptides in the brain. This work employs a completely novel electrochemical approach to monitor sub-second fluctuations of tyrosine-containing opioid peptides, such as methionine-enkephalin (M-ENK), in rat brain tissue. By combining multiple scan rate voltammetry with constant-potential amperometry in every voltammetric sweep, we have measured putative M-ENK fluctuations in intact and 6-OHDA lesioned rat striatum (male) in response to treatments that elicit robust DA fluctuations in striatal tissue (cocaine, L-DOPA, and unexpected food reward). Importantly, our electrochemical approach enables selective detection of chemical species within each scan, including striatal DA release. These measurements of opioid peptide dynamics will shed new light on the modulatory role of opioid peptides in a broad spectrum of pathological disorders related to dysfunction of the DA system.

Disclosures: C.A. Lee: None. **S.E. Calhoun:** None. **S.K. Smith:** None. **C. Meunier:** None. **M. Daush:** None. **G.S. McCarty:** None. **L.A. Sombers:** None.

Poster

636. Molecular Mechanisms of Cocaine Addiction

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 636.02/HHH10

Topic: G.08. Drugs of Abuse and Addiction

Title: Monitoring subsecond glucose dynamics in response to intravenous glucose and cocaine reveals spatially heterogeneous micro environments in the rat dorsal striatum

Authors: *S. SMITH, C. LEE, C. DOSTER, J. BAIRD, D. RAO, G. MCCARTY, L. SOMBERS;
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Abstract: Brain cells utilize glucose to fuel metabolic processes, and glucose consumption is increased upon neuronal activation. Real-time detection of glucose dynamics is imperative to understanding brain energy utilization and its involvement in neuropathological disorders, as well as the adaptations occurring upon exposure to substances of abuse. Extracellular glucose dynamics are dependent on two opposing forces: glucose availability (cerebral blood flow) and utilization. The location of intraparenchymal microvessels is heterogeneous and these are differentially activated in response to stimuli. Therefore, glucose dynamics are likely to be variable at the microscale. In the caudate putamen (CPu), dopamine-related neurochemical adaptations occur with many behavioral paradigms. However, far less is known regarding glucose signaling in this brain region. Attempts to assess glucose heterogeneity have been hindered by a lack of technology available for these measurements with sufficient spatiotemporal resolution. This work employs fast-scan cyclic voltammetry in conjunction with glucose-oxidase modified carbon fiber microelectrodes to monitor glucose dynamics with sub-second temporal resolution. We assessed heterogeneity in glucose signaling that occurred in response to intravenous administration of saline, glucose, and a cocktail of cocaine and raclopride. These investigations will advance our understanding of basic brain neuroenergetics, as well adaptations associated with substance abuse.

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Poster

636. Molecular Mechanisms of Cocaine Addiction

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Program#/Poster#: 636.03/HHH11

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA

NIMH

Title: Characterizing ΔFosB's transcriptional map in neuropsychiatric disorders using an HA-tagged transgenic mouse

Authors: *C. K. LARDNER, H. M. CATES, E. S. CALIPARI, E. A. RIBEIRO, J. FENG, E. J. NESTLER;
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Abstract: Neuronal gene expression mediates persistent changes in the function of neural circuits observed in many neuropsychiatric disorders. The progression of addiction, for example, begins with short-term molecular perturbations associated with brief exposure to a drug of abuse. If drug usage persists, these molecular changes initiate long-term alterations in gene expression that activate states of cellular and circuit activity unique to the addicted brain. One well-characterized regulator of this progression is ΔFosB, a Fos family transcription factor. ΔFosB is a splice variant of FosB, arising from a truncation which lends it unusual stability due to the loss of 3' degron domains. This allows for accumulation of the protein with continued exposure to a drug of abuse. ΔFosB plays a similar role in mediating adaptations to prolonged exposure to stress. Yet the scope and extent of ΔFosB's transcriptional activity has thus far been elusive due to the lack of antibodies suitable for chromatin immunoprecipitation (ChIP)-seq. Here, we characterize a transgenic mouse line in which *Fosb* is preceded by a hemagglutinin (HA) tag. As a result, ΔFosB is expressed with an HA tag fused at its N-terminus, allowing for anti-HA targeting of ΔFosB in a number of applications, including ChIP-seq. Specific targeting of ΔFosB *in vivo* will allow for elucidating its precise genetic targets and their corresponding molecular mechanisms in mediating the onset and persistence of addiction in addition to many other neuropsychiatric states.

Disclosures: C.K. Lardner: None. **H.M. Cates:** None. **E.S. Calipari:** None. **E.A. Ribeiro:** None. **J. Feng:** None. **E.J. Nestler:** None.

Poster

636. Molecular Mechanisms of Cocaine Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA R01-DA037170

NIDA F30-DA039637

Title: Sorting nexin 27 in midbrain dopamine neurons regulates addictive behavior to cocaine

Authors: *R. RIFKIN, M. S. FERNANDO, P. A. SLESINGER;
Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Natural rewards and addictive drugs increase the concentration of dopamine (DA) in the brain's reward circuit by altering the activity of dopaminergic neurons in the ventral tegmental area (VTA). Activation of G protein-gated inwardly rectifying potassium (GIRK or Kir3) channels provides an important inhibitory signal for regulating the excitability of DA neurons. Sorting Nexin 27 (SNX27), which is a component of the retromer forward trafficking complex, controls plasma membrane expression of GIRK channels in VTA DA neurons. We showed previously that loss of SNX27 in VTA DA neurons results in reduced $GABA_B$-GIRK currents. Here, we hypothesize that reduced $GABA_B$-GIRK currents in mesolimbic projecting DA neurons will increase the sensitivity of mice to cocaine-dependent locomotor sensitization. We crossed $SNX27^{fl/fl}$ mice with mice expressing Cre in DA neurons, TH_{BAC}-Cre mice, to construct a conditional knockout of SNX27 in DA neurons ($SNX27_{TH}KO$). To study locomotor sensitization, mice (male and female, ages 4-8 months) were acclimated in a locomotor activity chamber and injected (i.p.) daily with saline for three days. Mice were then injected (i.p.) daily with 3.75 mg/kg cocaine for 5 days. Locomotor activity was recorded during the 45 minutes after each injection. $SNX27_{TH}KO$ mice exhibited statistically significant greater locomotor sensitization. By contrast, control mice showed no sensitization with 3.75 mg/kg cocaine. In a separate cohort of mice, we quantified the amplitude of the $GABA_B$-GIRK currents in mesolimbic projecting DA neurons. To label these DA neurons in the VTA, we injected AAV5.EF1a.DIO.eYFP into nucleus accumbens (NAc) of $SNX27_{TH}KO$ and control mice. $GABA_BR$-GIRK currents were significantly reduced in NAc-projecting VTA DA neurons of $SNX27_{TH}KO$. To study the effect of rescuing the reduced $GABA_B$-GIRK currents in $SNX27_{TH}KO$ mice, we injected AAV5.EF1a.DIO.Girk2a-eYFP into the NAc of $SNX27_{TH}KO$ mice; Girk2a does not require SNX27 for plasma membrane expression. As expected, $GABA_B$-GIRK currents were restored in VTA DA neurons of $SNX27_{TH}KO$ mice injected with AAV5.EF1a.DIO.Girk2a-eYFP. We are currently examining the role of $GABA_B$-GIRK currents in the mesocortical and mesolimbic projecting DA neurons in the locomotor response to cocaine.

Taken together, these results reveal a novel pathway for reward processing involving SNX27 and GIRK channels in VTA DA neurons and highlight SNX27 as a new therapeutic target for treating addiction. Funding: NIH R01-DA037170 (PAS) and F30-DA039637 (RAR)

Disclosures: **R. Rifkin:** None. **M.S. Fernando:** None. **P.A. Slesinger:** None.

Poster

636. Molecular Mechanisms of Cocaine Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Title: Psychostimulant cocaine causes microvesicle release by activating sigma-1 receptor and ARF6

Authors: *Y. NAKAMURA, S.-Y. TSAI, T.-P. SU;
Cell. Pathobiology Section, Integrative Neurosci. Res. Br., NIDA IRP, Baltimore, MD

Abstract: The sigma-1 receptor (Sig-1R) is an endoplasmic reticulum (ER) chaperone which has been implicated in many central nerve system disorders including depression, neuropathic pain, and psychostimulant addiction. The Sig-1R is a pluripotent modulator that can modulate the functions of many proteins at the ER, plasma membrane, nucleus, and cytosol. Cocaine is a Sig-1R agonist that is known to affect the cellular localization of Sig-1R that plays an important role in cocaine addiction. Here we found that cocaine can dose-dependently (0.1, 1 and 10 μM) increase the Sig-1R in the extracellular space together with a concomitant increase of extracellular microvesicles in NG-108 cells. Pretreatment of cells with BD1063, a Sig-1R antagonist, completely blocks cocaine-induced release of microvesicles and Sig-1R. However, knockdown of Sig-1R causes an enhanced release of microvesicles. Interestingly, previous studies have also reported such a dilemma in which the Sig-1R antagonist decreased ethanol consumption (Sabino et al., Psychopharmacology (Berl). 2009) while deletion of the Sig-1R increased ethanol consumption (Valenza et al., Behav Brain Res. 2016). We examined next the effect of cocaine on the activity of ADP-ribosylation factor 6 (ARF6) which is a small GTP-binding protein known to be an important signaling molecule to regulate microvesicle release and membrane trafficking. Indeed, cocaine stimulation increases the activation of ARF6 in the form of ARF6-GTP. The exact mechanism on the cocaine-induced microvesicle and Sig-1R release and its relation to cocaine addiction is currently under investigation. (Supported by the IRP/NIDA/NIH/DHHS)

Disclosures: **Y. Nakamura:** None. **S. Tsai:** None. **T. Su:** None.

Poster

636. Molecular Mechanisms of Cocaine Addiction

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Title: eIF2α-mediated translational control regulates the persistence of cocaine-induced LTP in midbrain dopamine neurons

Authors: *A. PLACZEK[1,2], G. VIANA DI PRISCO[2], S. KHATIWADA[2], W. HUANG[2], K. KRNJEVIć[3], R. KAUFMAN[4], J. DANI[5], P. WALTER[6], M. COSTA-MATTIOLI[2]; [1]Mercer Univ. Sch. of Med., Macon, GA; [2]Baylor Col. of Med., Houston, TX; [3]McGill Univ., Montreal, QC, Canada; [4]SBP Med. Discovery Inst., La Jolla, CA; [5]Perelman Sch. of Med., Philadelphia, PA; [6]Univ. of California at San Francisco, San Francisco, CA

Abstract: Recreational drug use leads to compulsive substance abuse in some individuals. Studies on animal models of drug addiction indicate that persistent long-term potentiation (LTP) of excitatory synaptic transmission onto ventral tegmental area (VTA) dopamine (DA) neurons is a critical component of sustained drug seeking. However, little is known about the mechanisms regulating such persistent changes in synaptic strength in the VTA. We recently found that translational control by eIF2α phosphorylation (p-eIF2α) regulates the induction of cocaine-induced LTP in the VTA. We report that in mice with reduced p-eIF2α levels, cocaine induces persistent LTP in the VTA. Moreover, selectively inhibiting eIF2α-mediated translational control with the small molecule drug ISRIB, or by knocking down oligophrenin-1 (Ophn1)—an mRNA whose translation is controlled by p-eIF2α—in the VTA also prolongs cocaine-induced LTP. This persistent LTP is mediated by the insertion of GluR2 subunit-lacking AMPARs. Collectively, our findings suggest that eIF2α-mediated translational control regulates compulsive

drug seeking by acting as a defense mechanism that prevents the conversion from transient to persistent cocaine-induced LTP.

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Poster

636. Molecular Mechanisms of Cocaine Addiction

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Topic: G.08. Drugs of Abuse and Addiction

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NIH Grant F32 DA DA040414

Title: Mechanisms regulating protein synthesis in cultured nucleus accumbens neurons

Authors: ***M. E. WOLF**, M. T. STEFANIK;
Neurosci., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: The synthesis of new proteins is critical for the generation and maintenance of long-lasting synaptic modifications that lead to changes in behavior. Recent electrophysiological and biochemical work from our lab suggests that, during withdrawal from extended-access cocaine self-administration, adaptations in medium spiny neurons (MSNs) of the nucleus accumbens (NAc) leading to enhanced drug seeking are maintained by dysregulated local translation in the nucleus accumbens (NAc). As a first step towards understanding these mechanisms, we used fluorescent non-canonical amino acid tagging (FUNCAT) to visualize newly synthesized proteins in NAc MSNs.

Utilizing a co-culture system, PFC neurons obtained from P1 offspring of homozygous enhanced cyan fluorescent protein (ECFP)-expressing mice were co-cultured with NAc MSNs obtained from P1 Sprague-Dawley rats. MSNs in this co-culture system express high levels of high-conductance, calcium-permeable AMPA receptors, recapitulating the state of the NAc after incubation of cocaine craving. Direct detection of de novo protein synthesis was achieved with a strategy that utilizes FUNCAT and subsequent click-chemistry fluorescence tagging for visualization of newly translated proteins. Fluorescence immunocytochemistry was used to simultaneously quantify expression of specific proteins of interest in these cells.

Initial results have confirmed that the FUNCAT signal is nearly eliminated by an inhibitor of

protein translation (cycloheximide) and have shown, using immunohistochemistry, that dendritic sites of active protein translation co-localize with ribosomal subunits. Ongoing studies are utilizing a number of pharmacological tools in order to examine the regulation of protein synthesis by AMPARs, NMDARs, and group I mGluRs.
These results will further the understanding of mechanisms controlling the regulation of local protein synthesis in the NAc under normal conditions, how such regulatory mechanisms may differ between the NAc and other brain regions, and how they may be altered in pathological scenarios like addiction.

Disclosures: M.E. Wolf: None. **M.T. Stefanik:** None.

Poster

636. Molecular Mechanisms of Cocaine Addiction

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Program#/Poster#: 636.08/HHH16

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant K99DA038110

Title: Repeated restraint stress exposure during early withdrawal accelerates incubation of cue-induced cocaine craving

Authors: *J. A. LOWETH[1], S. CHAKROBORTY[1], A. R. WEST[1], J. A. ROSENKRANZ[2], M. E. WOLF[1];
[1]Neurosci., [2]Cell. & Mol. Pharmacol., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: A major challenge for treating cocaine addiction is the propensity for abstinent users to relapse. Two important triggers for relapse are cues associated with prior drug use and stressful life events. To study their interaction in promoting relapse during abstinence, we used the incubation model of craving and relapse in which cue-induced drug seeking progressively intensifies ("incubates") during withdrawal from extended-access cocaine self-administration. Our recent behavioral results show that exposure to repeated but not acute restraint stress during the first two weeks of withdrawal accelerates the initial rate of incubation of cue-induced cocaine craving. These data indicate that chronic stress exposure during early withdrawal facilitates incubation of cocaine craving, which is thought to contribute to enhanced relapse vulnerability. Previous studies have shown that chronic stress exposure enhances excitatory drive to the basolateral amygdala (BLA), a region critical for behavioral responses to stress. Given that glutamate projections from the BLA to the NAc are critical for incubation of cocaine craving, we

hypothesized that cocaine withdrawal and chronic stress exposure produce a synergistic increase in BLA neuronal activity which facilitates incubation of craving. To assess this, we are investigating how repeated restraint stress during early withdrawal from extended-access cocaine self-administration impacts BLA projection neuron activity. Using *in vivo* extracellular recordings from anesthetized rats, we will compare neuronal activity between the following four groups: cocaine self-administration + control conditions, cocaine self-administration + stress, saline self-administration + control conditions, saline self-administration + stress. Changes in the firing rate of BLA neurons and in the number of spontaneously active neurons in the BLA will be assessed. In this way, we can assess the effects of cocaine exposure alone on BLA physiology, as well as the interaction between both cocaine and chronic stress exposure. These findings may identify potential mechanisms by which stress enhances cue-induced relapse vulnerability in abstinent cocaine addicts.

Disclosures: **J.A. Loweth:** None. **S. Chakroborty:** None. **A.R. West:** None. **J.A. Rosenkranz:** None. **M.E. Wolf:** None.

Poster

636. Molecular Mechanisms of Cocaine Addiction

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA015835

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Title: Extended-access cocaine self-administration leads to increased GluN3-containing NMDA receptor function in the rat nucleus accumbens

Authors: *D. T. CHRISTIAN, K. Y. TSENG, M. E. WOLF;
Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: Cue-induced cocaine craving intensifies or incubates during withdrawal from extended-access cocaine self-administration. After prolonged withdrawal, the expression of incubated cocaine craving is mediated by GluA2-lacking, Ca2+-permeable AMPARs (CP-AMPARs) that accumulate in the nucleus accumbens (NAc). NMDARs play a major role in Ca2+ signaling, but little is known about how NMDAR transmission may be altered during incubation of cocaine craving. However, recent studies have shown that incorporation of GluN3-

and GluN2B-containing NMDARs accompanies cocaine-induced increases in CP-AMPAR levels in the ventral tegmental area (Yuan et al, 2013). To determine if NMDAR transmission in the NAc is altered following extended-access cocaine self-administration, we utilized whole-cell patch clamp electrophysiology in NAc medium spiny neurons (MSN). Specifically, we measured evoked NMDAR-mediated synaptic responses across a range of membrane holding potentials (-80 through +40 mV; 20 mV steps). We found that cells from cocaine exposed rats during "late" withdrawal (WD>35) exhibited a significant increase in NMDAR currents at both negative (-80,-60, -40 mV) and positive (+ 40mV) membrane holding potentials when compared to saline control cells. Such shifts in the current-voltage relationship are consistent with the incorporation of GluN3 subunits into functional NMDARs. We also examined cocaine exposed animals during an earlier withdrawal period (WD13-20) which precedes the synaptic incorporation of CP-AMPARs into excitatory synapses onto NAc MSNs. Again, significant increases in NMDAR currents at both negative (-80,-60,-40 mV) and positive (+40 mV) membrane holding potentials when compared to saline control cells were found, indicating the presence of GluN3-containing NMDARs. We are currently evaluating potential GluN3-selective antagonists to examine the specific role of GluN3-containing NMDARs in the incubation model. Overall, these data indicate that alterations in NMDAR function in the NAc, likely mediated by incorporation of GluN3-containing receptors, precede the incorporation of CP-AMPARs during withdrawal from extended-access cocaine self-administration, and are then maintained throughout the period when incubated drug seeking is maximal. Support: DA015835, DA009621 and postdoctoral NRSA DA36963 (D.T.C.).

Disclosures: D.T. Christian: None. **K.Y. Tseng:** None. **M.E. Wolf:** None.

Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA015835

Title: Nucleus accumbens core miRNA expression after the incubation of cocaine craving

Authors: M. E. WOLF, *C. MURRAY;
Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: In a rat model of cocaine addiction, cue-induced cocaine craving intensifies or "incubates" during withdrawal from extended-access cocaine self-administration. Cocaine

craving is mediated in part by AMPAR transmission onto medium spiny neurons (MSN), the principal cell type in the nucleus accumbens (NAc). During incubation, incorporation of Ca^{2+}-permeable AMPARs (CP-AMPARs) strengthens NAc synapses, while group I mGluR-mediated synaptic depression shifts from an mGluR5-dependent mechanism that is expressed presynaptically to an mGluR1-dependent mechanism that is expressed postsynaptically by the removal of CP-AMPARs. Previous work from our lab has shown that these synaptic adaptations depend upon ongoing protein translation for their maintenance, and therefore may involve regulation by microRNAs (miRNAs), a class of small non-coding RNAs that repress mRNA translation. The goal of this study is to identify miRNAs that are differentially expressed in the NAc core after incubation of cocaine craving, and manipulate miRNA expression in vitro and in vivo to functionally characterize the role that specific miRNAs play in cocaine-dependent synaptic adaptations during withdrawal. We are particularly interested in miRNAs that regulate AMPAR subunit expression. To this end, rats self-administered saline or cocaine (6 h/d for 10 d) and were killed after 50 days of withdrawal. RNA isolated from the NAc core was assessed using the miRCURY LNA 7th generation microRNA microarray platform (Exiqon), which contains capture probes for all known rat miRNA. Based on these findings, we plan to manipulate the affected miRNAs within cell culture to assay potential effects on AMPAR expression on NAc neuronal processes and examine the functional correlates of the miRNAs in the incubation model in vivo. Support: DA015835

Disclosures: M.E. Wolf: None. **C. Murray:** None.

Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R01DA037257

NIGMS GM09545902

Title: Examination of E3 ubiquitin ligases in cocaine-induced plasticity in the nucleus accumbens during withdrawal following extended-access cocaine self-administration

Authors: *C. T. WERNER[1], J. A. MARTIN[1], L. E. MUELLER[1], Z.-J. WANG[2], A. CACCAMISE[1], A. F. STEWART[1], R. L. NEVE[3], D. M. DIETZ[1];
[1]Pharmacol. and Toxicology, [2]Physiol. and Biophysics, The State Univ. of New York at Buffalo, Buffalo, NY; [3]Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Drug addiction is a chronic brain disease that develops gradually in response to compulsive use of psychoactive substances. These drugs, including cocaine, cause progressive, drug-dependent alterations in brain regions associated with reward and motivation, including the nucleus accumbens (NAc). These neuroadaptations, which include changes in spine morphology, gene expression and protein expression, facilitate craving evoked by drug-associated cues that underlie persistent relapse susceptibility. The ubiquitin-proteasome system (UPS), which is responsible for proteasomal-dependent protein degradation, has been shown to be involved in cocaine conditioned place preference (CPP) and cue-induced seeking following extended-access self-administration. However, how ubiquitin protein (E3) ligases, which polyubiquitinated proteins for proteasomal degradation, regulate cocaine plasticity has not been studied. Here, we examined E3 ligases Trim3, which polyubiquitinates gamma actin to regulate spine architecture, as well as Smurf1, which polyubiquitinates some members of transforming growth factor (TGF)-β superfamily involved in transcription. Following extended-access self-administration, we found dynamic changes in Trim3 and Smurf1 in the NAc that differed in subcellular fractions and were dependent on withdrawal. We will use viral and pharmacological approaches to characterize the function of Trim3 and Smurf1 in the NAc in cocaine-dependent neuroadaptations and relapse-like behaviors.

Disclosures: C.T. Werner: None. **J.A. Martin:** None. **L.E. Mueller:** None. **Z. Wang:** None. **A. Caccamise:** None. **A.F. Stewart:** None. **R.L. Neve:** None. **D.M. Dietz:** None.

Poster

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Support: NIH R01 DA037257

NIGMS GM09545902

Title: Activin A is increased in the hippocampus following prolonged, but not acute, withdrawal from extended-access cocaine self-administration and mediates cue-induced reinstatement

Authors: *L. MUELLER[1], C. T. WERNER[1], Z.-J. WANG[2], J. A. MARTIN[1], A. CACCAMISE[1], A. F. STEWART[1], D. M. DIETZ[1];
[1]Pharmacol. and Toxicology, The State Univ. of New York At Buffalo, Buffalo, NY; [2]Physiol. and Biophysics, The State Univ. of New York at Buffalo, Buffalo, NY

Abstract: Risk of relapse in cocaine addiction is often attributed to craving evoked by cues previously associated with drug. Relapse vulnerability persists even after prolonged periods of drug abstinence due to progressive, drug-dependent plasticity that occurs in brain structures involved in reward, learning and memory, including the nucleus accumbens (NAc) and hippocampus (HPC). Drug-induced plasticity includes, among others, changes in spine morphology, protein expression and gene expression. The transforming growth factor (TGF)-β superfamily is a family of multifunctional proteins that regulate a wide variety of cellular responses, including gene expression. Recent studies have shown transcription factor SMAD3 and homodimer protein Activin A, both members of the TGF-β superfamily, and BRG1, a chromatin remodeler that forms a complex with SMAD3, are important for relapse-like behaviors following cocaine exposure in animal models of addiction. However, the role of TGF-β signaling in the HPC in drug addiction has yet to be examined. Here, we show that following extended-access cocaine self-administration, Activin A is increased in the HPC, but not the NAc, after a cue-induced reinstatement test on withdrawal day (WD) 30 but not WD1. Intra-HPC infusions of antibody directed at Activin A prior to testing on WD30, but not WD1, attenuated cue-induced reinstatement compared to vehicle controls. Importantly, cue-induced reinstatement was still reduced in antibody-treated animals when tested the following day. Together, these results suggest Activin A signaling in the HPC is increased following prolonged, but not acute, withdrawal from extended-access cocaine self-administration and is required for expression of cue-induced reinstatement.

Disclosures: L. Mueller: None. **C.T. Werner:** None. **Z. Wang:** None. **J.A. Martin:** None. **A. Caccamise:** None. **A.F. Stewart:** None. **D.M. Dietz:** None.

Poster

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Support: NIDA R00 DA031790

NIDA T32 DA07244

Title: Hypermethylation of the glutamate transporter-1 gene in the nucleus accumbens after long access cocaine self-administration and withdrawal

Authors: *R. KIM, K. J. REISSNER;
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Abstract: Relapse to drug abuse is associated with alterations in glutamatergic signaling within the nucleus accumbens (NAc). One particularly salient feature of these alternations is decreased glutamate uptake. The astroglial glutamate transporter, GLT-1, accounts for the majority of glutamate clearance from the synapse and thus, plays an important role in glutamatergic signaling. Drugs of abuse, including cocaine, significantly decrease GLT-1 protein expression in the NAc. Interestingly, this decrease is correlated with both degree of cocaine exposure and length of withdrawal. We have found that GLT-1 protein and mRNA levels in the NAc are differentially regulated based on exposure and withdrawal time, indicating multiple mechanisms of regulation. While short-access self-administration is sufficient to suppress protein levels, a significant decrease in GLT-1 mRNA is observed in the NAc only after long access cocaine self-administration and withdrawal. The goal of the current study is to examine the epigenetic mechanism(s) responsible for the decrease in GLT-1 mRNA following withdrawal from long access cocaine self-administration. Male Sprague-Dawley rats were trained to self-administer i.v. cocaine or saline for 6 hours per day for 10 days. Immediately following the last day of cocaine self-administration, rats remained abstinent in the home cage for 45 days. Twenty-four hours following the last day of withdrawal, rats were sacrificed and tissue was harvested from the NAc. Using an antibody against 5-methylcytosine, methylated DNA was immunoprecipitated. The amount of GLT-1 methylation was then quantified using quantitative PCR and primers specific to GLT-1. Results indicate that long access cocaine self-administration and withdrawal results in an approximately three fold increase in GLT-1 gene methylation over rats that self-administered saline. This hypermethylation of the GLT-1 gene in rats exposed to long access cocaine followed by withdrawal suggests transcription suppression is responsible for the observed decrease in GLT-1 mRNA. Future studies will examine chromatin modification and transcription factors that may also contribute to the observed decreases in GLT-1 mRNA after long access cocaine self-administration and withdrawal.

Disclosures: **R. Kim:** None. **K.J. Reissner:** None.

Poster

636. Molecular Mechanisms of Cocaine Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH (DA-027115)

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Title: Cocaine self administration alters dna methylation, hydroxymethylation, and mrna expression in rats

Authors: *K. PLOENSE[1], P. VIEIRA[2], L. BUBALO[2], G. OLIVARRIA[2], A. E. CARR[1], T. E. KIPPIN[2];
[1]Univ. of California Santa Barbara, Goleta, CA; [2]Univ. of California Santa Barbara, Santa Barbara, CA

Abstract: Cocaine addiction is a chronic disorder that involves excessive, uncontrolled drug consumption. Both humans and rodents will escalate the amount of cocaine taken when it is readily available indicating that excessive intake is dependent on prior consumption. In rodents, however, escalation is not observed when access is restricted to "limited" (i.e. 1 hr) daily sessions. Here, we investigated whether control over drug intake or drug exposure are the critical factors in escalation by employing a mixed limited-contingent exposure and prolonged noncontingent exposure model. . Rats were implanted with a permanent jugular catheter and then allowed to lever press to self-administer (FI20 with a 20 s light cue paired with each infusion) saline vehicle (0.1 ml/infusion) or cocaine (0.25 mg/infusion) under 3 conditions: limited-access (1 h/ day), extended-access (6 h/day), and 1 h limited-access + 5 h non-contingent exposure (via yoking to extended access rats) to cocaine. Based on the first 10 min and first hour of daily access, we observed rapid escalation of cocaine intake in both the extended-access and limited-access + non-contingent conditions (ps < 0.05). We also observed a delayed escalation of cocaine intake in the limited-access condition within the first 10 min of self-administration ($p < 0.05$), but not across the 1 h of cocaine availability. Interestingly, escalation of cocaine intake was accelerated in the limited + non-contingent-access condition relative to the extended-access condition. However, relative to the other cocaine conditions, the limited-access + non-contingent group exhibited markedly more non-reinforced responses indicating that distinct behavioral mechanisms drive escalation by contingent versus noncontingent drug exposure (ps<0.05). Additionally, post-mortem quantification of *homer2* (a gene implicated in cocaine intake and associated) mRNA expression within the dmPFC indicated elevation only in the extended-access conditions ($p<0.05$). Rises in *homer2* mRNA were associated with distinct levels of DNA methylation, hydroxymethylation, and transcription factor binding to the *Homer2* gene. Together, these findings indicate that either contingent or non-contingent "excessive" cocaine exposure supports escalation but have differential effects on the temporal patterning of operant responsiveness as well as molecular correlates of escalation.

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Poster

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Title: Cell signaling pathways involved in the cocaine-induced increase in mu opioid receptor expression in PC12 cells

Authors: K. THERIAULT, F. LERI, *B. E. KALISCH;
Univ. Guelph, Guelph, ON, Canada

Abstract: Cocaine abuse has been linked to elevated mu opioid receptors (MOR) at the protein and mRNA level in key regions of the brain involved in craving and drug seeking. *In vitro* and *in vivo* studies have determined that nitric oxide (NO) and multiple signaling pathways, including phosphatidyl inositol-3 kinase (PI3K)-Akt, protein kinase C (PKC), and mitogen activated protein kinase (MAPK) pathways are involved in cocaine-induced increases in MOR expression. Furthermore, our laboratory established that in PC12 cells, NO modulates the activation of the Akt, MAPK, and PKC pathways. Whether there is an interaction between NO and these signaling pathways in cocaine-induced MOR expression in this cell line is not known. The objective of the current *in vitro* study was to investigate the role of NO in the modulation of these signaling pathways in cocaine-induced increases in MOR expression. To assess the activation of the PKC, Akt, and MAPK pathways by cocaine, PC12 cells were administered repeated intermittent treatments of cocaine (100 μM) and protein was isolated 15 minutes, 30 minutes, 1 hour, 2 hours, or 4 hours after treatment. Phosphorylated PKC, Akt, and MAPK protein levels were evaluated using western blotting. It was found that cocaine significantly activated the PKC and MAPK pathways following 30 minutes and 2 hours of treatment, respectively. To determine the modulation of these pathways by NO, cells were pre-treated with the NO synthase (NOS) inhibitor, N^{ω}-nitro-L-arginine methylester (L-NAME; 20 mM). Protein was isolated at 30 minutes and 2 hours to assess the impact of NO on the PKC and MAPK pathways, respectively. Additional data on the effects of L-NAME on these pathways will be available at the meeting. These studies will clarify the cellular effects that cocaine has on opioid systems, and may identify NO-regulated pathways as a potential target for pharmacotherapies for cocaine addiction.

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Poster

636. Molecular Mechanisms of Cocaine Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Title: Sigma-1 receptor regulates cocaine-induced serotonergic action via monoamine oxidase B expression

Authors: ***Y. KIMURA**[1], A. F. HOFFMAN[2], C. R. LUPICA[2], T.-P. SU[1];
[1]Cell. and pathology section, Integrative Neurosci. Res. Br., [2]Electrophysiology Res. Section, Integrative Neurosci. Res. Br., IRP NIDA/NIH, Baltimore, MD

Abstract: The action of cocaine on dopaminergic neurons in the ventral tegmental area (VTA), and its contribution to the addictive properties of this drug have been well characterized. By inhibiting the dopamine transporter (DAT) present on axon terminals of VTA dopamine (DA) neurons, cocaine elevates extracellular dopamine DA levels. However, since the rewarding actions of cocaine are not eliminated by DAT knock down, this suggests that there are still other crucial mechanisms underlying the rewarding effects of cocaine. In this study, we explored the possibility that cocaine, through the ER chaperon sigma-1 receptor (Sig-1R) may activate VTA DA neurons by altering the expression of monoamine oxidase B (MAOB) in the upstream serotonergic neuron that terminates on GABAergic neuron near the VTA. We previously demonstrated that cocaine can suppress the gene transcription of MAOB by translocating Sig-1R to the nuclear envelope to recruit chromatin-remodeling factors that suppress the MAOB promoter. MAOB is known to be expressed selectively in the serotonergic neuron and not in any other monoaminergic neurons. In this study we confirmed a strong MAOB expression in serotonergic neurons in mouse brain. We further show that Sig-1R and MAOB are co-localized in serotonergic neurons within the dorsal raphe (DR). We examined if the expression of MAOB in DR serotonergic neurons differs between wild type vs Sig-1R knockout mice. We also examined whether the Sig-1R and MAO-B positive serotonergic neurons in DR projects to GABAergic neurons which in turn projects to DA neurons within the VTA. This Sig-1R-mediated neuronal pathway implies the crucial involvement of serotonergic neurons in the action of cocaine. These results reveal a new molecular mechanism underlying the actions of cocaine which may contribute to a better understanding of the addictive properties of this widely abused drug. (This study supported by the IRP/NIDA/NIH/DHHS)

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Poster

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Support: KAKEN 15K01837

KAKEN 24591735

Title: Nrf exacerbates cocaine addiction after social defeat stress

Authors: *Y. N. OHNISHI[1], Y. KAWAHARA[2], Y. H. OHNISHI[2], R. NEVE[3], E. J. NESTLER[4], A. NISHI[2];
[2]Pharmacol., [1]Kurume Univ. Sch. of Med., Kurume, Japan; [3]Viral Gene Transfer Core, McGovern Inst. for Brain Res. at MIT, Cambridge, MA; [4]Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Generally, mental stress enhances irresistible impulse to addictive craving. However, the molecular mechanism is not fully understood. We reproduced this behavior in a mouse model, in which pre-exposure of mice to social defeat stress (SDS) increased cocaine preference. Motivation for preference is well known to be controlled by dopamine neurotransmission in reward system network from the ventral tegmental area to nucleus accumbens (NAc). We therefore performed microdialysis to detect dopamine levels in the NAc. Dopamine levels in the NAc increased when mice were placed in cocaine-conditioned box, and the increase in dopamine levels was significantly enhanced after SDS. As dopamine is known to be an inducer of oxidative stress, we tried whether antioxidant-rich-foods or anti-oxidative reagent decreases cocaine preference after SDS, the former had no effect, but the latter had a trend to decrease the preference and locomotor sensitization by cocaine. Next, we measured the expression level of oxidative stress response proteins, and found that nuclear respiratory factor (Nrf) was significantly increased only when mice were treated with cocaine after SDS. Furthermore, overexpression of dominant negative form of Nrf in NAc suppressed cocaine preference after SDS. Now we examine whether the anti-oxidant reagent inhibits the enhanced expression of Nrf after cocaine administration following by SDS.

Disclosures: **Y.N. Ohnishi:** None. **Y. Kawahara:** None. **Y.H. Ohnishi:** None. **R. Neve:** None. **E.J. Nestler:** None. **A. Nishi:** None.

Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: FRQS

Collectivité Territoriale de Martinique

Title: N-acetylcysteine decreases the motivation to self-administer cocaine in rats

Authors: *R. HODEBOURG, A.-N. SAMAHA;
Dept. of Pharmacol., Univ. of Montréal, Montréal, QC, Canada

Abstract: Chronic cocaine intake decreases basal levels of glutamate in brain regions that regulate reward, such as the nucleus accumbens. This effect correlates with the vulnerability to relapse in rats and is thought to involve downregulation of the cysteine/glutamate antiporter located on glial cells. In cocaine-experienced rats, systemic administration of the cysteine prodrug N-acetylcysteine (NAC), restores the function of the cysteine/glutamate exchanger and reduces the vulnerability to relapse. Drug addiction involves several behavioural symptoms that involve relapse to drug use during abstinence, but also an increased motivation to take drug. Indeed, spending much time and effort obtaining, consuming and recovering from drugs is one of the diagnostic criteria for addiction. Our objective here was to determine whether systemic administration of NAC influences the motivation to take cocaine in cocaine-experienced rats. To this end, rats were trained to press a lever to self-administer cocaine (0.25 mg/kg), during daily 6-h sessions. Cocaine was available intermittently during each session. Intermittent drug intake, in contrast to continuous drug intake, is more effective in producing brain and behavior changes that are relevant to addiction (Zimmer et al., 2011) and is also thought to best model the way experienced cocaine users take the drug (Beveridge et al., 2012). Thus, in a protocol adapted from Zimmer et al. (2011), cocaine was available in 6-min bins, separated by 26-min time-out periods, for 6h/day. First, we determined whether acute administration of NAC (0, 30, 60, 90 mg/kg, ip) influences the motivation to self-administer cocaine, or food, as assessed using a progressive ratio schedule of reinforcement. Acute administration of 30, 60 and 90 mg/kg NAC decreased the motivation to take cocaine without affecting the motivation for food. Next, we determined whether chronic NAC administration (0, 60 mg/kg, i.p) during withdrawal from self-administered cocaine influences the later motivation to take the drug. NAC injected daily during either 7 or 14 days of withdrawal from cocaine had no effect on the motivation to take the drug. Finally, we examined the effect of NAC treatment prior to each cocaine self-administration session on the later motivation to take cocaine. NAC treatment reduced both cocaine intake during the intermittent-access sessions and the later motivation to take cocaine. Thus, acute

treatment and chronic pretreatment with NAC are both effective in decreasing the motivation for cocaine. These findings contribute to a growing literature suggesting that NAC deserves clinical attention as a treatment for cocaine addiction.

Disclosures: R. Hodebourg: None. **A. Samaha:** None.

Poster

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Title: AMPK in the NAc regulates cocaine reinforcement and morphological plasticity

Authors: *Y. HAN[1,2,3], L. YI-XIAO[4], Y.-X. XUE[3], Z.-B. DING[3], J. SHI[3], L. LU[2,3,5];
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Abstract: Repeated cocaine exposure causes compensatory neuroadaptations in neurons of the nucleus accumbens (NAc), a region known to mediate the reinforcing effects of drugs. Recent studies have suggested that the cellular energy sensor AMPK regulates neuronal morphology and membrane excitability. However, the potential involvement of AMPK in cocaine addiction is still unknown. Here we found that intravenous cocaine self-administration induced a significant decrease in AMPK activity persisting for at least 7 d of withdrawal in the NAc shell. Through direct genetic and pharmacological manipulations of AMPK signaling, we found that

augmenting AMPK activity in NAc shell markedly attenuated cocaine reinforcement of self-administration behavior, while inhibition of AMPK activity significantly enhanced cocaine reinforcement, as indicated by downward or upward shifts in fixed ratio dose-response curves. Inhibiting AMPK activity in NAc shell also increased motivation for cocaine injections on more demanding progressive ratio schedules. These behavioral findings were associated with changes in the phosphorylation of AMPK and CRTC1, and expression of CREB-dependent genes and membrane GluR1 and GluR2 in the NAc shell. Moreover, the effects of AMPK on cocaine reinforcement and motivation were prevented by expressing constitutive active CRTC1 or knockdown of CRTC1 in the NAc shell. We also found that AMPK mediated cocaine-induced dendritic spine morphology of NAc neurons. Altogether, our results indicate that AMPK regulates cocaine reinforcement and motivation through long-term remodeling of neuronal morphology and function in the NAc via transcriptional reprogramming, and its activity during cocaine use influences the development and persistence of addictive behaviors.

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Poster

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8G12-MD007600

Title: PKCι/λ and PKMζ protein expression in mesocorticolimbic areas during cocaine behavioral sensitization

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Abstract: Cocaine addiction induces long-lasting alterations in the mesocorticolimbic system, some of which may be mediated by the mechanisms of long-term potentiation (LTP). Persistent phosphorylation by protein kinase M zeta (PKMζ), a brain-specific atypical isoform of PKCζ, mediates late-LTP maintenance. This notion was recently challenged when PKC/PKMζ knock-out mice showed intact LTP and reversal of LTP by infusion of its inhibitor ZIP. This suggested that LTP can develop independently of PKMζ, and ZIP may block another atypical PKC. PKCι/λ is another atypical PKC isoform whose marked homology (88%) to PKMζ makes it a potential candidate for participation in the LTP process when PKMζ is not present; and could possibly compensate for PKMζ loss in the knockout mice. Here, we aim to investigate the role PKMζ, and PKCι/λ may play in cocaine-induced LTP, using the cocaine behavioral sensitization model. To this end, we examined the total protein expression profile of PKMζ and PKCι/λ in naïve animals and at different time points of the cocaine behavioral sensitization: 24 hours after an acute exposure, 24 hours after 5 days of cocaine sensitization, and 24 hours after a 7 day withdrawal period following 5 days of cocaine sensitization. Sprague Dawley male rats (250g) received intraperitoneal cocaine (15mg/kg) or 0.9% saline injections for 1 or 5 consecutive days, and locomotor activity was recorded for 1hr. The rats were sacrificed and tissue micro punches of the ventral tegmental area (VTA), nucleus accumbens (NAc), prefrontal cortex (PFC), and hippocampus (Hipp) were subjected to protein extraction and western blot analysis. Results show no significant difference in the expression of PKCι/λ and PKMζ between all four brain areas of naïve animals. After a single cocaine exposure, PKCι/λ protein expression was decreased in the PFC. In cocaine sensitized animals PKMζ protein expression and not PKCι/λ was significantly increased in the NAc and Hipp. After a 7 day withdrawal period, PKCι/λ protein expression was significantly decreased in the hippocampus. These results suggest that PKMζ protein increase in NAc might precede important changes for the expression of sensitization; possibly playing a role in NAc LTP. PKC ι/λ protein expression decrease in the PFC after an acute cocaine exposure and after a 7 day withdrawal period may result from a compensation to increased PKMζ activity. Future measurement of phosphorylated PKCι/λ and PKMζ (activated state) will allow us to ascertain their role in cocaine sensitization and LTP formation. This may shed light into the pathological mechanisms of cocaine-related plasticity during the addiction process.

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Poster

636. Molecular Mechanisms of Cocaine Addiction

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

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Topic: G.08. Drugs of Abuse and Addiction

Support: NSF 105-1353263

Title: Contribution of estrogen receptor subtypes to the effect of estradiol on cocaine-induced dopamine release in the nucleus accumbens

Authors: *K. E. YOEST[1], J. A. CUMMINGS[1], B. J. ARAGONA[1,2], J. B. BECKER[1,3,2]; [1]Dept of Psychology, [2]Neurosci. Program, [3]Mol. and Behavioral Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI

Abstract: There are well established sex differences in the susceptibility to addiction, where women show faster escalation of drug use and are more prone to relapse than their male counterparts. Similar findings have been reported in rodent models, where females acquire cocaine-taking behavior more rapidly than males. Gonadal hormones modulate this sex difference, as the positive subjective effects of psychomotor stimulants are enhanced by estradiol in women, and estradiol also enhances the acquisition of cocaine self-administration in female rats. Our laboratory has previously reported that acute estradiol administration also enhances cocaine-induced dopamine release in the nucleus accumbens (NAc) shell in females but not males (Yoest et al. Estradiol rapidly enhances dopamine in nucleus accumbens shell of female but not male rats. Program No. 617.12. 2014 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience, 2014. Online.) However, which estrogen receptor is mediating this effect is still unknown. The estrogen receptors ERα and GPER-1 are both expressed in the NAc (Almey et al., Hormones & Behavior, 74:125-138, 2015) and reduction of ERα in this area blocks estradiol induced place preferences (Walf et al., Neuropsychopharmacology, 32:522-530, 2007). However, ERβ mediates the effect of estradiol on cocaine-induced reinstatement (Larson & Carroll, Neuropsychopharmacology, 32:1334-1345, 2007), suggesting a potential role for this receptor as well. With this experiment, we tested the effect of acute selective estrogen receptor activation on cocaine-induced dopamine release in the NAc shell. Gonadectomized male and female rats were anesthetized and treated with either the ERα selective agonist propyl pyrazole triol, the ERβ selective agonist diarylpropionitrile, the GPER-1 selective agonist G1, or vehicle 30 min prior to administration of cocaine (10mg/kg i.p.). Fast scan cyclic voltammetry (FSCV) was used to measure stimulated dopamine release in the NAc shell both prior to and after cocaine administration. We found that activation of specific estrogen receptor subtypes differentially modulate dopamine release in the presence of cocaine. These findings further elucidate the mechanism by which estradiol exerts sex-specific effects on dopamine release. Importantly, dopamine signaling in the NAcc has been implicated in the acquisition and escalation of drug taking behaviors, aspects of addiction that are more pronounced in female addicts. A full understanding of the mechanism by which estradiol may render women more susceptible to compulsive drug use will help to develop gender-specific treatments in clinical settings.

Disclosures: K.E. Yoest: None. **J.A. Cummings:** None. **B.J. Aragona:** None. **J.B. Becker:** None.

Poster

636. Molecular Mechanisms of Cocaine Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant 1R25MH092912-01

Title: Intranasal OT reduces cocaine conditioned locomotion and elicits changes in endocannabinoid receptors within the mesolimbic system

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Abstract: Oxytocin (OT) is a neuropeptide secreted by the hypothalamic paraventricular nucleus (PVN) and commonly associated with social behaviors, stress responses and drug-addiction. Previous studies have shown that OT has anxiolytic properties associated with cues in a cocaine-conditioning paradigm, but the underlying mechanism remains unknown. This study aims to characterize possible mechanistic interactions between OT and the endocannabinoid system mediating cocaine conditioning and anxiety response, in particular the cannabinoid receptor type 1 (CB1) and the transient receptor potential vanilloid type-1 (TRPV1). Rats were exposed to activity chambers after receiving systemic intraperitoneal injections of cocaine (10 mg/kg) or saline 0.9%. On the last day (D7), rats received intranasal infusions of OT (1 ug/uL) or vehicle 30 minutes prior being exposed to the cue-associated environment. Our results showed that OT pretreatment reduced cocaine-paired conditioned locomotion. Preliminary western blot analysis showed an upregulation of OT receptors within the hippocampus and amygdala and a downregulation of these receptors within the prefrontal cortex (PFC). Further immunohistochemical analysis will be conducted to determine possible interactions or colocalization between OT, CB1 and TRPV1 receptors. This preliminary data suggests intranasal OT as a novel therapeutic approach of cocaine addiction.

Disclosures: G.C. Molina-Castro: None. **A.N. Figueroa-González:** None. **A.J. Loyola-Vélez:** None. **E. Torres-Hernández:** None. **C.S. Maldonado-Vlaar:** None.

Poster

636. Molecular Mechanisms of Cocaine Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: NCCR Synapsy

Title: Disrupting astrocyte-neuron lactate transfer persistently reduces conditioned responses to cocaine

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Abstract: Drug memories that associate contextual cues with the effects of drugs are known to shape persistent drug seeking behaviors in rodents. In abstinent humans, drug cues are known to evoke salient, persistent and overwhelming memories of drug-taking experiences, thereby inducing higher risks of craving and relapse. Since the transfer of glycogen-derived lactate from astrocytes to neurons is required for long-term memory, we explored the possibility that disrupting glycogenolysis in the basolateral amygdala (BLA) could impair the acquisition and maintenance of positive affective memories associated with cocaine-associated cues. We have observed that rats treated with intra-BLA infusions of the inhibitor of glycogen phosphorylase (DAB 300 pmol/side), 15 minutes before conditioning sessions of a conditioned place preference (CPP), failed to exhibit a clear cut preference for side previously paired with cocaine. To assess the importance of astrocyte-derived L-actate in the maintenance of cocaine- induced CPP, another group of rats received a post-conditioning administration of DAB into the BLA prior to the test. Whereas saline treated rats exhibited a strong preference for the rewarding compartment up to seven days, DAB-treated rats only transiently displayed an abolished preference for the cocaine compartment that was restored after seven days. In contrast, a double injection of DAB (15 min before and 5 hrs after contextual re-exposure) completely abolished the cocaine attractiveness for up to two weeks. Importantly, rats injected with DAB in home cages 24h prior to the test exhibited a strong CPP, suggesting a role of DAB on experience-dependent memory reconsolidation. Finally, we demonstrated that drug memory was rescued by Lactate co-administration through a mechanism requiring the synaptic plasticity related transcription factor Zif268, and extracellular signal-regulated kinase (ERK) signalling pathway. Interestingly, co-administration of DAB and Pyruvate failed to do so, demonstrating that Lactate played a non-metabolic role in this process. We then targeted the prefrontal cortex (PFC) with a similar protocol, but rats continued to exhibit a strong preference for the cocaine compartment. Taken

together, these results highlight a signaling role of astrocytic lactate in both acquisition and maintenance of cocaine-seeking behavior and open novel therapeutic avenues to reduce the long-lasting impact of drug cues on conditioned responses to cocaine.

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Poster

637. Human Cognition and Memory III

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Topic: H.02. Human Cognition and Behavior

Support: ERC-StG 261177

NWO-Vidi 452-12-009

NWO Zwaartekracht 024.001.006

Title: Tracking the emergence of hierarchical concept representations

Authors: *S. THEVES, D. NEVILLE, J. BELLMUND, G. FERNÁNDEZ, C. F. DOELLER; Donders Institute, Radboud Univ., Nijmegen, Netherlands

Abstract: Concepts provide the scaffolding structures to interpret the world and assign meaning to novel information. This often requires incorporating relations between knowledge entities at multiple hierarchical levels. While previous fMRI studies revealed that hippocampal and prefrontal activity scales with the acquisition of simplistic one-dimensional concepts, it remains elusive how the brain accumulates and represents knowledge at multiple levels of abstraction. Here we combined model-based fMRI with representational similarity analysis to examine local computations during learning of a two-level hierarchical category structure and their relation to neural concept representations. During scanning, participants learned to categorize artificial complex stimuli into two basic and four sub-categories according to a hierarchical rule. In each trial incremental feedback indicated up to which level in the hierarchy the participants placed a stimulus correctly. In addition to these feedback-guided learning trials, we interleaved feedback-free probe trials to track the transfer of conceptual knowledge to novel information. The amount of level-specific conceptual knowledge per trial was estimated from behaviour during learning and transfer trials in a hierarchical state-space model and regressed against brain activity to track the accumulation of hierarchy-specific conceptual knowledge. We find that behavioral performance on both category levels increased across trials and ultimately exceeded chance-level

in all participants. Importantly, the learning trials on the basic category level preceded the learning trials on the subcategory level, confirming the hierarchical nature of the acquisition process. In addition, participants acquired explicit knowledge of the categorization rules as assessed in a final debriefing questionnaire. Preliminary analyses of fMRI data indicate level-dependent involvement of striatal, prefrontal and hippocampal structures during acquisition. Before and after learning, exemplars of each category were presented in task-independent blocks to assess learning-induced changes in multi-voxel pattern similarity across exemplars. Preliminary results point towards a representational clustering of exemplars according to their position in the acquired concept structure. Finally, we aim to investigate interactions between neural concept representations, learning-related activity, and behavior. In sum, this study could shed new light on the representational dynamics underlying concept formation at different levels of abstraction.

Disclosures: S. Theves: None. **D. Neville:** None. **J. Bellmund:** None. **G. Fernández:** None. **C.F. Doeller:** None.

Poster

637. Human Cognition and Memory III

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 637.02/HHH33

Topic: H.02. Human Cognition and Behavior

Title: Item-specific representation during episodic memory encoding and retrieval

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Abstract: Contemporary models of episodic memory posit that remembering involves the reenactment of encoding processes. Although encoding-retrieval similarity (ERS) has been consistently reported and linked to memory success, the nature of the neural pattern reinstatement is poorly understood. Using high-resolution fMRI, our results obtained clear evidence for item-specific pattern reinstatement in the frontoparietal cortex (PFC) but not in the ventral visual cortex (VVC), even when the encoding-retrieval pairs shared no perceptual similarity. Nevertheless, the brain regions and voxels carrying item-specific representation differed significantly between encoding and retrieval, and the item-specificity for ERS was smaller than that for encoding or retrieval. Cross-region representation analysis suggests that the encoded representation in the VVC was reinstated in the FPC during retrieval. These results suggest retrieval might involve the reinstatement of a more abstract representation of the

encoded information, which emphasizes the constructive nature of memory retrieval that helps to serve important adaptive functions.

Disclosures: **G. Xue:** None. **X. Xiao:** None. **R.A. Poldrack:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Title: Investigating the components of episodic memory in humans: can they be mapped using electrophysiology?

Authors: *L. A. DE STEFANO, S. E. RHOTEN, M. J. WENGER, E. J. ZOCCOLI, M. G. APPEL, S. M. TOFTELY, S. A. WETMORE, S. D. GRONLUND;
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Abstract: Episodic memory retrieval relies on integration of information about what an item was, as well as when and where it was encountered. Previous research from animal lesion studies using a novelty preference paradigm found that the hippocampus and the medial prefrontal cortex were critical for different aspects of recollection of episodic events (DeVito & Eichenbaum, 2010). The present study attempts to adapt this paradigm to assess these components behaviorally and physiologically in humans using a memory recognition task. Participants studied two virtual "rooms" containing images of women, with each image presented consecutively in a random location for 4s. Between rooms, participants engaged in a go/no-go distractor task for either 15 seconds ("short" retention interval) or 3 minutes ("long"). After both rooms were studied, participants made old/new judgments with half of the items being new. Half of the images appeared in the same location as during the study phase ("stationary") with the others appearing in a new location ("displaced"). Behavioral results showed significantly longer reaction times (RTs) for new items than old, and longer RTs for long retention intervals relative to short. Electrophysiological (EEG) results show a significant main effect of old/new status in the left parietal area in the interval from 500 to 800 ms post-stimulus onset, with old items eliciting larger amplitudes than new items, but this result was unaffected by retention interval. Additionally, the effects associated with the "when" and "where" aspects of remembered events were analyzed, both with respect to their individual contributions to performance and the integration of all three aspects. Finally, trial-by-trial regression analyses were performed with results providing additional insight into the temporal evolution of all three informational components of episodic memory.

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Poster

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Topic: H.02. Human Cognition and Behavior

Title: Event related potentials during memory retrieval processes for fractal images in a paired-associate paradigm

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Abstract: The ability to remember relies on a complex interplay of processes that we are only beginning to understand. Event-related potential (ERP) studies have proven to be powerful tools in the delineation of these processes. Nevertheless ERP components related to memory are often hard to interpret due to the nature of the stimuli (e.g. words or faces), and the memory task (e.g. retrieval vs recognition). We wondered whether a given known ERP component might in fact reflect an effective-retrieval process which is not camouflaged by another non-specific component. This study sought to solve this issue by using artificial fractal images as stimuli and by comparing two tasks which were almost the same in every aspect except the memory process. In the Delayed Match to Sample task (DMS) subjects had to pair a cue stimulus with itself (control task) and in the Delayed Paired Association task (DPA) subjects had to pair the cue stimulus with its pair associate (previously learned). Twenty students (10 women, $\bar{x}$= 23.45 ± 3.73 years) participated in the experiment. All subjects performed above 80% during the DPA learning task. Mean percentage of correct responses were 98.94 ± 1% in DMS and 96.54 ± 3.88% in DPA. Response times of correct answers were significantly faster in the DMS (680 ± 215 ms) as compared to DPA (938 ± 406 ms). EEG Activity was recorded from 30 electrodes. ERPs mean voltage were analyzed on six regions of interest, each containing the average value of a group of four electrodes and grouped between the two hemispheres as following: left anterior (F7, F3, FC5, FP1), left central (FC1, C3, CP5, T7), left posterior (CP1, P7, P3, O1), right anterior (F4, F8, FC6, Fp2), right central (FC2, C4, CP6, T8), and right posterior (CP2, P4, P8, O2). We asked which memory-related ERP component explained the differences between the two tasks. Three ERP components, P300 (200-350ms), N400 (450-600ms) and Positive Slow Wave (700-850ms) with distinct scalp topographies, were found to differ significantly among the

two tasks. These electrophysiological differences are interpreted as reflections of processes that correlated with memory retrieval processes. This approach, utilizing stimuli with a very small verbal contamination and comparing a memory task with a control task, strengthen our understanding of the ERP component implicated in memory retrieval processes.

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Poster

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Canada Institutes of Health Research (CIHR) Grant

Title: Successful memory encoding is independently predicted by slow and fast modulations of oscillatory alpha power in humans

Authors: *S. SUN[1,2], J. S. CANT[2], S. FERBER[1,3], C. J. HONEY[1];
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Abstract: Successful memory encoding appears to be associated with changes in electrophysiological rhythms (oscillations). However, in the case of memory for visual stimuli, both increases and decreases in parieto-occipital alpha band (8-12 Hz) power, as measured in human magneto/electroencephalography (M/EEG), have been linked to successful subsequent memory retrieval. Here we seek to explain these varying results, by considering the timing of the oscillatory changes, and the broader connections of alpha power with both mnenomnic and perceptual processing. Traditionally, increases in alpha power ("alpha synchronization") are thought to reflect an attenuation of bottom-up sensory processing. Thus, we hypothesized that peri-stimulus decreases in alpha power may support initial stimulus encoding by amplifying sensory input, while post-stimulus increases in alpha power may support short-term consolidation by reducing interference. In a test of this hypothesis, participants were presented

with a continuous streams of words, followed by old/new recognition tests. We recorded EEG during the presentation phase, and then contrasted the spectral signatures associated with successful encoding (hits) versus later forgetting (misses). We found that peri-stimulus decreases in alpha power over occipito-parietal sites during the presentation phase were related to successful encoding. This is consistent with the interpretation that alpha desychronization supports the initial processing of the to-be-encoded stimulus. Furthermore, we also found that post-stimulus increases in alpha power were predictive of successful encoding. This suggests that once the information has entered the system, suppression of additional incoming sensory input promotes the consolidation of information into a durable memory trace. Subsequent analyses showed that these opposing effects in the alpha band might operate at different timescales, and are independently and additively predictive of memory outcomes. These results offer a novel framework for interpreting the role of fluctuating alpha oscillations in the ongoing formation of immediate percepts and longer-term memories.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: VA CSR&D CDA Program #CX000516

VA RR&D Merit Review #RX001612

Title: Working memory network integrity in Veterans with a history of repeated blast-related mTBI: A resting state fMRI study

Authors: ***K. F. PAGULAYAN**[1,2], D. G. COOK[1,2], M. REILLY[2], E. PESKIND[1,2], N. KLEINHANS[2];
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Abstract: Repeated mild traumatic brain injury (mTBI) is common among military personnel who served in the conflicts in Iraq and Afghanistan. A significant number of Veterans who sustained repeated blast-related mTBIs report ongoing cognitive difficulties, and there is emerging evidence that blast-related mTBI can be associated with alterations in brain structure and function that are evident years post injury. Although working memory difficulties are frequently reported in this population, the neural underpinnings of this particular cognitive

inefficiency remain unclear. In order to address this question, this study evaluated intrinsic functional connectivity within the working memory network in Operation Iraqi Freedom (OIF)/Operation Enduring Freedom(OEF)/Operation New Dawn (OND) Veterans with a history of repeated blast-related mTBI (N=25) and deployed controls (N=17). Average time since injury in the mTBI group was 71 months (range 18-109 months). All participants completed a neuropsychological measure of working memory called the Auditory Consonant Trigrams Test (ACT) and two 6.5 minute resting state fMRI scans. A subset of participants in the mTBI group (N=21) also completed a self-report measure of current cognitive functioning called the Behavior Rating Inventory of Executive Function-Adult Version (BRIEF-A). fMRI analyses were conducted from frontal seed regions selected because they are well-established components of the working memory network. A high percentage (62%) of Veterans with a history of blast-related mTBI reported clinically significant difficulties with working memory on the BRIEF-A, but no group differences were present on the ACT, an objective measure of working memory. The fMRI results revealed increased connectivity from the frontal seed regions to areas in the frontal-temporal-parietal regions of the brain in the mTBI group relative to the deployed controls. Further, within the mTBI group, but not the deployed control group, performance on the ACT was correlated with increased connectivity between the frontal seed regions and key components of the working memory network including superior parietal lobe and cerebellum. These correlations were present after controlling for PTSD and age, and support the possibility that long-term alterations in the working memory network may result from repeated blast-related mTBI.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: DoD NDSEG

Title: Acute stress-induced cortisol elevation enhances memory consolidation of highly similar items

Authors: ***A. JIANG**[1], T. TRAN[1], F. MADISON[2], A. BAKKER[3];
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Maryland, Col. Park, Baltimore, MD; [3]Dept. of Psychiatry and Behavioral Sci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: It is well-established that stress-induced changes in cortisol significantly affect memory function. Cortisol has been shown to enhance memory consolidation, the process of converting short-term memories into long-term memories, and impair memory retrieval, the process of accessing previously stored long-term memories. However, little is known about the effect of stress-induced changes in cortisol on the hippocampus and its subregions, which have recently been shown to contribute dissociable computational functions to the consolidation and retrieval of memories. In the present study, we examined the effect of acute stress, induced by the Trier Social Stress Test (TSST), on a memory task designed to tax the functioning of the dentate gyrus and CA3 subregions of the hippocampus. The study aimed to determine whether stress-induced cortisol differentially affects hippocampus-dependent memory consolidation and retrieval processes in healthy young adults. Sixty-nine young adults completed a two-day study in which subjects either underwent the TSST immediately following the encoding part of the memory task, or immediately prior to the recognition part of the memory task on the second day. Control subjects completed the same study procedures but underwent a control version of the TSST that did not induce a stress response. Stress-induced elevation of cortisol during memory consolidation enhanced the ability to discriminate between highly similar items 24 hours later while stress-induced elevation of cortisol during memory retrieval had no significant effect on memory performance. These findings show that acute stress-induced change in cortisol differentially affects the consolidation and retrieval stages of memory function, and particularly enhances the consolidation of highly similar items, a function thought to rely strongly on the dentate gyrus and CA3 subregions of the hippocampus.

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Poster

637. Human Cognition and Memory III

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Topic: H.02. Human Cognition and Behavior

Support: MOH | National Medical Research Council (NMRC) - NMRC/STaR/015/2013

Title: Napping vs. revising for long-term retention of declarative memories

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Abstract: Sleep is crucial to learning and memory consolidation. It is well established that daytime naps provide benefits to declarative memory retention, relative to an equivalent period of wakefulness. However, it is unclear whether the enhanced retention associated with napping outweighs the benefits of revising learned information instead of sleeping. Clarity on this point is essential to assess the usefulness of napping to educators and students. Participants learned detailed conceptual knowledge during several learning sessions across a day. Between these sessions they were allowed a one-hour daytime nap (nap; n=23), a rest period where they remained awake (wake; n=24), or they revised previously learned information (revise; n=25). We found that revising and napping resulted in significantly better memory than wakefulness when tested immediately after learning that day. Moreover, after one week, memory in the nap group remained significantly superior to the wake group, while the enhancement observed in the revise group was no longer significant. Thus, despite the revise group having an additional hour with the learning materials, the benefits of this extra time were not as long lasting as those offered by a nap. This provides a powerful demonstration of the benefits of napping for long term retention of declarative memories, and suggests daytime naps could benefit learning in young adults.

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Poster

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Title: Medial PFC supports successful recall and recognition of schema-congruent information

Authors: *E. A. WING, V. IYENGAR, R. CABEZA;
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Abstract: A recently proposed theoretical framework postulates that there is a push-and-pull relationship between the medial PFC—thought to facilitate the encoding of material congruent

with preexisting schema—and subregions of the MTL—which may preferentially encode novel or schema-incongruent material. Past research pertaining to this framework has naturally focused primarily on the encoding phase. Less is known therefore, about the retrieval processes that operate on congruent/incongruent information encoded through potentially dissociable mechanisms, or about the interaction between encoding and retrieval processes in this context. To help clarify how schema congruence influences mnemonic processes within and across phase, we examined the brain activity both during the encoding of schema congruent and incongruent word-scene pairs and also at a subsequent cued-recall phase in which subjects received the word cue and attempted to retrieve visual details of the corresponding (semantically congruent or incongruent) picture. Behaviorally, we found a schema-congruency enhancement effect whereby visual memory was more vivid for schema-congruent images. A similar enhancement was also observed in a separate test of conceptual (vs. perceptual) memory. Neuroimaging contrasts showed that activity in both medial PFC and MTL increased along with visual memory ratings for schema-congruent pairs, but for the recall of incongruent images, this memory-activity relationship was selective to MTL. Additionally, we found an interaction in medial PFC whereby activity also tracked subsequent forced-choice scene recognition performance, but only for scenes congruent with retrieval cues. Ultimately, these findings add to a growing body of research exploring the relationship between episodic memory and prior knowledge, and suggest that various forms of episodic retrieval for information differing in schema-congruence may rely on mechanisms that are sensitive to semantic relationships when information is initially processed.

Disclosures: E.A. Wing: None. **V. Iyengar:** None. **R. Cabeza:** None.

Poster

637. Human Cognition and Memory III

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 637.10/III1

Topic: H.02. Human Cognition and Behavior

Support: Polish National Science Centre Grant 2013/10/E/HS6/00186

Title: Psychophysiological correlates of visual working memory enhancement in video game players.

Authors: *A. BRZEZICKA[1,2], P. DOBROWOLSKI[3], J. MICHALAK[1], M. SKORKO[3], B. SOBCZYK[1], N. JERMAKOW[1];
[1]Univ. of Social Sci. and Humanities, Warsaw, Poland; [2]Cedars-Sinai Med. Ctr., Los Angeles, CA; [3]Polish Acad. of Sci., Warsaw, Poland

Abstract: Visual working memory (VWM), or the ability to store and maintain visual information over short periods of time, is essential for acquiring and processing information. Although this system is crucial for everyday functioning, it is also very limited in capacity. Finding a way to extend this capacity is potentially beneficial for almost all cognitive (and probably not only) processes. One common daily routine that has been shown to improve VWM capacity is video game playing (in comparison studies: Blacker & Curby, 2013; Wilms, Petersen, & Vangkilde, 2013; as well as in a training study: Blacker et al., 2014). The observed performance advantage of video game players (VGPs) over non-players (NVGPs) was also shown to be correlated with anatomical changes in the brain (Tanaka et al., 2013). In our study we compared VGPs playing in different game genres on their VWM performance using two indices of its capacity: the behavioral estimate of VWM capacity (*K* scores) based on change detection paradigm performance and contralateral delay activity (CDA) indexing VWM based on changes in EEG signal recorded during task completion. 45 participants were recruited via online questionnaire: 15 NVGPs, 15 real-time strategy players (defined as playing RTS games > 5 hours per week) and 15 first-person shooter players (defined as playing FPS games > 5 hours per week). All participants were male and the mean age was similar across groups. Each participant underwent 576 trials of the change detection task (144 per load, using load 2, 3, 4 and 5). CDA was derived from parietal electrode clusters (O1, P3, PO3, P7 vs. O2, P4, PO4, P8) by subtracting averaged ipsilateral signal from signal contralateral to presented stimuli.
On the behavioral level, RTS players had higher capacity (*K* scores) than NVGPs, especially at higher loads. They were also better than FPS players, although these differences were smaller (significant set size x group interaction: $F(4.08,85.81) = 3.10$, $p = .019$, $\eta_p^2 = .129$). Psychophysiological results mimicked the behavioral. RTS players had larger CDA compared to FPS and NVGP groups, especially at load 2, where RTS players had larger CDA than did both NVGPs ($p = .016$) and FPS players ($p = .024$) and load 3, where RTS players had larger CDA than NVGPs, $p = .027$. Interestingly, averaged CDA signal was found to correlate with average *K*, but for the NVGP group only ($r = -.599$, $p = .030$). Our results show that cognitive enhancement observed in video game players has a clear psychophysiological correlate. They also support the thesis that VGPs, but especially RTS players, are able to process a larger number of representations in their VWM, which can influence the course of other cognitive functions.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIMH Grant 5F32MH102009

Title: Chunking as a rational strategy for lossy data compression in visual working memory tasks.

Authors: *M. R. NASSAR[1], M. J. FRANK[2];
[1]Metcalf Hall, [2]CLPS, Brown Univ., Providence, RI

Abstract: Humans are capable of representing recent visual information in a robust but flexible form of working memory. The amount of information that can be stored in working memory is inherently limited, although the nature of limitations has been a subject of intense debate [1,2]. Both sides of that debate have relied heavily on models that assume independent item encoding. While recent work has highlighted item dependencies that are elicited by data compression strategies that exploit stimulus statistics, it is unknown whether such dependencies are in play for standard working memory tasks that involve unpredictable and independent stimuli [3,4]. Here we combine computational modeling with human experiments to show that under such conditions stimulus data can be, and is, compressed through joint encoding of similar feature values. To do so, we first develop a toy model of capacity-limited memory storage and find that simulated task performance is improved when stimulus values for similar items are stored as a single chunk. The simulated performance bonus for chunking was highly contingent on the statistics of each stimulus array, which could be summarized by a statistical measure of stimulus clustering. Second, we show that human subjects performing a delayed-estimation task are more accurate and confident when reporting colors from more clustered stimulus arrays. The observed performance bonus was not driven by an increase in perfect color reproductions, but rather by an increase in reproductions slightly offset from the target color, consistent with chunking. Furthermore, the bonus was larger on trials following positive feedback, suggesting a potential role for evaluative systems in the chunking process. Taken together, these findings suggest that visual item encoding is subject to a rational chunking process that sacrifices encoding precision and item independence to maximize performance in situations where capacity limits are small relative to the amount of visual information available. We consider mechanisms whereby such optimization could be achieved through efficient gating of feature representations into prefrontal memory stores.

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Disclosures: **M.R. Nassar:** None. **M.J. Frank:** None.

Poster

637. Human Cognition and Memory III

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Program#/Poster#: 637.12/III3

Topic: H.02. Human Cognition and Behavior

Title: Priming facilitates pattern separation processes

Authors: D. SANDBERG, S. D. GALE, *B. KIRWAN;
Psychology, Brigham Young Univ., Provo, UT

Abstract: Pattern separation is a computational process whereby potentially similar memory representations are made as dissimilar as possible at the time of encoding. The complementary process, pattern completion, is the process whereby memory representations are retrieved given a noisy or degraded cue. Previous neuroimaging research in humans has demonstrated that the hippocampus is especially prone to perform pattern separation while the adjacent medial temporal lobe (MTL) cortex is biased toward pattern completion. Further research with patients with hippocampal damage has demonstrated a specific impairment in pattern separation in the context of spared recognition memory. The mnemonic similarity task used to assess pattern separation processes relies on explicit memory processes. However, it is unclear if implicit memory representations have an influence on pattern separation and pattern completion processes. This study used masking to present stimuli at intervals below conscious perception (<30ms) in order to measure the potential effects of implicit representations on pattern separation and pattern completion. We hypothesized that priming would facilitate pattern separation dependent mnemonic discrimination. We modified the mnemonic similarity task in which participants view novel, repeated (targets) and perceptually/conceptually similar stimuli (lures) and indicate whether images are new, old, or similar, respectively. Prior to each memory probe stimulus, we presented a masked prime for 8, 24, or 100ms. Primes preceding targets and lures were either the exact same as the memory probe stimulus or the related lure stimulus. Primes preceding novel stimuli were randomly selected from a large pool of unrelated foils. Exposure to the 24ms prime facilitated mnemonic discrimination performance when the prime matched the memory probe but interfered with mnemonic discrimination when there was a mismatch. These data indicate that priming facilitates pattern separation processing.

Disclosures: D. Sandberg: None. **S.D. Gale:** None. **B. Kirwan:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: Google Faculty Research Award

NSF Grant CNS-1422831

Title: Evidence for consolidation of pattern-separated memories in ventromedial prefrontal cortex but not in hippocampus

Authors: *D. K. BJORNN[1], A. L. HOWELL[2], B. B. ANDERSON[3], A. VANCE[3], C. B. KIRWAN[4];
[1]Psychology, [2]Neurocience Ctr., [3]Information Systems, [4]Psychology & Neurosci. Ctr., Brigham Young Univ., Provo, UT

Abstract: Pattern separation is a computational process in which similar memory traces are encoded as dissimilar as possible in order to reduce overlap. The hippocampus has been shown to be critical for this process. While there is considerable research on pattern separation over single study sessions, little is known about how these memory representations are consolidated over several days. The present study sought to examine the pattern separation process over a five-day period using functional magnetic resonance imaging (fMRI).
Fifteen participants studied images of everyday objects as they were presented one at a time on a computer screen and were instructed to indicate whether the item is generally found indoors or outdoors. After study, participants completed fMRI scans once a day for five days in which images were presented and participants again indicated whether the item is generally found indoors or outdoors. Images were composed of three categories, namely, exact repeats of studied items, items similar to studied items, and completely new images. We took repeat and similar images for each day from a different subset of the original studied images so no images were presented more than once during the scan. New images were used as a functional baseline.
A whole-brain analysis revealed a significant interaction between day and stimulus type in the ventromedial prefrontal cortex (vmPFC). Specifically, activity for repeat items increased linearly across days while activity for lure items remained near baseline. A comparison between repeat items for the vmPFC and hippocampus regions showed a significant day by region interaction, with vmPFC activity increasing for each subsequent day while hippocampal activity remained near baseline.
Both the hippocampus and vmPFC are important structures in long-term memory. Prior studies have shown that activity in the vmPFC increases as memories are consolidated and our results are consistent with these findings. Our results also give evidence for differing processes in the

consolidation of pattern-separated memory representations. While activity in the hippocampus is not affected over several days, an increase is observed in the vmPFC. Further research can focus on closer examination of the patterns of activity in hippocampal subregions over time.

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Poster

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Program#/Poster#: 637.14/III5

Topic: H.02. Human Cognition and Behavior

Title: The effect of false recognition on memory encoding and discrimination

Authors: *N. MUNCY, B. KIRWAN;
Neurosci., Brigham Young Univ., Provo, UT

Abstract: Pattern separation is the process whereby distinct memory representations are encoded in such a way as to reduce potential overlap. Pattern completion is the complementary processes whereby memory representations are retrieved when given a partial or noisy retrieval cue. Behaviorally, pattern separation and pattern completion have been tested using mnemonic discrimination paradigms that employ either a study/test or continuous recognition format. Correct rejection of new but perceptually similar stimuli (i.e., Lure CRs) are thought to depend on accurate pattern separation processes whilst false alarms to lures (Lure FAs) are thought to reflect pattern completion processes. What has yet to be determined is whether or not Lure FAs result in a rewriting of the original memory trace or in the formation of a new memory trace that is distinct from the original. Participants studied 200 visual stimuli while performing an incidental encoding task. During the first test phase, participants were presented stimuli that were either the same as, or similar to stimuli previously seen, and the participants indicated whether the stimuli were the same or similar. This resulted in false alarm (Lure FA), correct rejection (Lure CR), hit, and miss responses. In a second surprise recognition memory test, participants were presented both the original and similar stimulus simultaneously in a two-alternative forced-choice format. High- resolution whole-brain functional MRI (fMRI) data were collected during both testing phases. Behavioral analyses indicated that second-test performance following first-test false alarms was essentially at chance; participants responded to previous false alarms with either a hit or false alarm equally (FA-FA vs. FA-Hit). Likewise, confidence ratings and response times for both FA-FA and FA-Hit were indistinguishable. FMRI activation in the left inferior frontal and supramarginal gyri during first-test FA responses predicted a second-test

correction from pattern completion to recognition (FA-Hit); that is, differential activity in these regions during first-test FAs were predictive of continued pattern completion (FA-FA) or correction (FA-Hit) in the second test. These data indicate that these regions are resilient to incorrect pattern completion.

Disclosures: **N. Muncy:** None. **B. Kirwan:** None.

Poster

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Program#/Poster#: 637.15/III6

Topic: H.02. Human Cognition and Behavior

Title: An fMRI analysis of visual unitization and the age-related associative memory deficit

Authors: ***M. B. MEMEL**, L. RYAN;
Psychology, Univ. of Arizona, Tucson, AZ

Abstract: Healthy aging impairs associative memory -- the ability to remember associations between previously unrelated pieces of information (Naveh-Benjamin, 2000). Unitization, the process of creating one semantic representation that includes both items in an associative pair, attenuates age-related associative deficits (Zheng, et al., 2015; Bastin et al., 2013; Ahmad et al., 2014). Examples of unitized pairs include compound words (Giovanello et al., 2006; Quamme, et al., 2007) and words imagined in a specific color (Diana et al., 2011). Many studies have argued that unitizeable associative pairs rely less on hippocampal function than traditional associative pairs. For example, hypoxic patients, with damage relatively limited to the hippocampus, benefit from word pairs that can be unitized (Quamme et al., 2007). Thus, it is believed that with verbal stimuli, unitization of associative pairs allows for memory based on non-hippocampal medial temporal structures. Less is known about the benefit of unitization with visual stimuli, such as objects and scenes. Objects and scenes are two types of visual stimuli frequently encountered in the world and perceived in relation to one another. In the present study, we manipulated the degree of visual integration between objects and scenes, by presenting objects either next to their paired scene (Separated condition) or embedded within their paired scene (Combined condition) and measured associative memory performance in young and older adults while undergoing fMRI.
Based on previous studies of unitization, we hypothesized that older adults would receive a mnemonic benefit from the integration of visual associative pairs. Further, we predicted greater hippocampal activation during the encoding of Separated associative pairs than Combined pairs, due to a greater need for binding between items. Consistent with our hypotheses, visual

integration of objects and scenes improved associative memory performance in older adults. In addition, hippocampal activation was greater during the Separated than Combined condition across age groups. However, the degree of hippocampal activation during the Separated condition was related to performance in young, but not older adults. In older adults, the perirhinal cortex was the only medial temporal region that significantly predicted performance. These findings may suggest that hippocampal activation is less successful in older adults than young. Alternatively, object processing, which relies on the perirhinal cortex and is impaired in some older adults (Ryan et al., 2012) may be a greater predictor of successful associative memory with object-scene pairs.

Disclosures: **M.B. Memel:** None. **L. Ryan:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: VA MERIT Award RX000825

Title: Effects of acute enhancement of cortical dopamine on cognitive control and associated neural function in mTBI Veterans

Authors: ***M. E. BALLARD**[1,2], T. A. VEGA[2], A. S. KAYSER[1,2];
[1]UCSF, San Francisco, CA; [2]VA Northern California Hlth. Care Syst., Martinez, CA

Abstract: Fifteen percent of mild TBI (mTBI) patients report persistent cognitive problems, including difficulty remembering and focusing attention. Still, there are few effective medications for treating cognitive problems in TBI patients. In this study, we set out to test whether the FDA-approved medication tolcapone, by selectively enhancing cortical dopamine tone, could improve working memory in combat Veterans with mTBI. To date, 10 Veterans with mTBI have been administered a single oral dose of 200mg tolcapone and placebo on separate sessions, in counter-balanced order, under double-blind conditions. Nine of the subjects then completed an emotional facial working memory task in the MRI scanner; the tenth subject completed the task outside the scanner. In each trial, subjects first encoded either one (low load) or three (high load) neutral or fearful faces. Then, during a delay period, they identified an arousing or neutral distractor as a face or scene. Finally, they indicated whether a presented probe face was shown during the encoding period. As expected, participants' recognition memory was less accurate in the high (vs. low) load condition (0.64 vs. 0.80, $t_9 = 7.28$, $p <$

0.0005) and when remembering fearful (vs. neutral) faces (0.70 vs. 0.74, $t_9 = 3.11$, $p = 0.013$), collapsed across drug conditions. Consistent with previous work, a larger distractor cost (i.e., lower memory accuracy) was found with congruent (face) versus incongruent (scene) distractors, collapsed across valence and drug conditions (0.70 vs. 0.73, $t_9 = 2.23$, $p = 0.052$). At the neural level, the task robustly activated the ventral visual stream and a frontoparietal network. In preliminary results, part of the frontoparietal attention network (including the right inferior parietal lobule) tended to be more active during encoding in the high (vs. low) load condition, collapsed across treatments ($p < 0.01$, uncorrected). Notably, ROI analyses revealed a significant effect of drug condition on activity in the bilateral fusiform face area (FFA) during cue encoding in the high versus low load conditions ($p \leq 0.015$), and this effect was in the same direction as a non-significant drug treatment effect on memory accuracy in the high load condition (0.62 vs. 0.66, $p = 0.18$). These findings provide preliminary evidence that the task is a useful tool for measuring acute drug effects on working memory maintenance and resistance to distraction in veterans with mTBI. Our ongoing work seeks to further elucidate the ways in which tolcapone can influence cognitive control and its neural correlates, with particular attention to functional connectivity between prefrontal and relevant higher order sensory areas.

Disclosures: M.E. Ballard: None. **T.A. Vega:** None. **A.S. Kayser:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: Templeton foundation

Title: The strategic allocation of working memory and episodic memory in prospective remembering: A neural network model

Authors: M. TOMOV[1], I. MOMENNEJAD[2], K. NORMAN[2], *J. D. COHEN[3,2];
[1]Dept. of Psychology and Ctr. for Brain Sci., Harvard Univ., Boston, MA; [2]Princeton Neurosci. Inst., [3]Princeton Univ., Princeton, NJ

Abstract: The successful realization of future plans (prospective memory or PM) requires the agent to either maintain, or be able to retrieve, a goal for execution at a future time. This must be accomplished over periods during which the agent is involved in other, ongoing activities, while at the same time remaining responsive to the circumstances that demand goal execution. There are at least two distinct memory strategies that can be used to solve the PM problem: active

monitoring, which relies on working memory (WM) to maintain the goal and a preparatory attentional process to continuously monitor the environment for events that signal the opportunity for goal execution; or episodic encoding and retrieval, which initially commits the goal to episodic memory (EM) and relies on automatic retrieval at the opportunity for goal execution arises. The WM strategy relies on a system widely considered to be capacity limited, and thus can incur costs with regard to other ongoing tasks that compete for this capacity. In contrast, the EM strategy carries a greater risk of retrieval failure, and thus PM failure. Each of these strategies may be better suited to some circumstances than others and, in general, PM success may rely on striking the right balance between them. Here we describe a computational model that incorporates neural network mechanisms previously used to implement working memory (Leaky Competitive Accumulators, c.f. Usher and McClelland 2001) and episodic memory, and integrates these to address a variety of behavioral findings previously reported from five seminal experiments on PM by Einstein et al. (2005): the effect of target focality, PM task priority, different monitoring strategies, the cost of increasing the number of goal states or PM targets, and the aftereffects of PM intentions. The model offers novel predictions that remain to be behaviorally tested, and we discuss ways in which it can be extended to address additional PM phenomena, as well as a broader class of task switching effects.

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Poster

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Topic: H.02. Human Cognition and Behavior

Title: The influence of context elements on pattern separation

Authors: *A. HEDGES, B. KIRWAN;
Psychology, Brigham Young Univ., Provo, UT

Abstract: The memory of an item and its contextual information when it was encountered is defined as contextual memory. Researchers have proposed that the relationship of objects and their contexts is at the core of long-term declarative memory, converging on hippocampal processing. To further explore the effect of surrounding visual context on memory, this experiment evaluated behavioral performance in contextual memory task that especially taxes pattern separation processes. Most current studies of pattern separation use stimuli of isolated objects on a white background. The current experiment used objects placed in front of a background that was either congruent with the object (e.g., a shopping cart set against a grocery

store) or incongruent with the object (e.g., a gorilla set against a classroom). Adding a contextual component to memory specificity tasks is believed to increase the level of difficulty of the task by interfering with the ability to successfully perform pattern separation processes. In addition, some researchers propose that when the object matches its context, there will be more efficient discrimination between stimuli and thus more accurate responses. However, some hypothesize that repeat and similar objects presented with the same background will be more accurately remembered than objects presented with different backgrounds due to the increase of interference.
In this experiment, 71 participants (40 females) performed a two-part memory task. In the first part (study phase), participants were shown 260 images of everyday objects with backgrounds that were congruent or incongruent. The images were presented for 2500 ms with an inter-trial interval of 500 ms. In the second part (test phase), participants were shown 320 images that contained items either exactly the same as or similar to the previously studied items (repeats and lures, respectively). These images were also presented for 2500 ms with a 500 ms inter-trial interval. During the test phase, participants were instructed to ignore the backgrounds and indicate whether the item in the middle of the image had been previously studied or similar to an object previously studied.
After performing an ANOVA, we found that correct "similar" responses to lure images (indicative of correct pattern separation) was facilitated by a similar, incongruent background. On the other hand, recognition was facilitated by a repeated, congruent background. Also, a difference between males and females was found, with males performing better with recognition but not pattern separation.

Disclosures: **A. Hedges:** None. **B. Kirwan:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: Emory University Seed Grant

Title: Dynamic neural correlates of overt and covert autobiographical memory retrieval

Authors: ***C. S. FERRIS**[1], C. INMAN[1], G. JAMES[2], S. HAMANN[1];
[1]Psychology, Emory Univ., Atlanta, GA; [2]Univ. of Arkansas for Med. Sci., Little Rock, AR

Abstract: Autobiographical memory (AM) retrieval is a complex process that recruits dynamically changing networks of brain regions as processing shifts between memory search, access, and content elaboration. In prior fMRI work we have used analyses to characterize whole-brain changes in dynamic connectivity during AM retrieval, highlighting time-varying engagement of the hippocampus, PFC, and other regions. Here we extended these investigations, using high temporal (1 second TR) and spatial (2 mm isotropic) resolution fMRI, an optimized experimental design, and both covert and overt (spoken) retrieval to test theoretical accounts of AM retrieval. Motion was minimal (<1 mm) for overt trials. After a low-level, arrow detection baseline, participants retrieved unrehearsed AMs to cue words across a long retrieval period, followed by ratings of vividness and emotion, with overt (spoken) retrieval during half of the trials. Importantly, overall movement during overt retrieval was not significantly different than movement covert retrieval. Relative to previous studies of AM retrieval, we found a similar network of activated brain regions during both the overt and covert AM retrieval, including the hippocampus, medial, and lateral PFC. A broadly similar core AM retrieval network was identified for covert and overt AM retrieval, with relatively greater hippocampal activation during memory access. Key brain activation differences between covert and overt AM retrieval included activation of speech comprehension and facial motor regions during overt AM retrieval. Across both overt and covert trials, dynamic changes in activation relative to the pre-cue baseline included hippocampal, anterior temporal, and posterior cingulate activation early in AM retrieval. During later periods of AM retrieval posterior involved in reconstruction of sensory details and fronto-parietal regions involved in working memory were activated greater than the pre-stimulus baseline. These findings provide evidence that accessing and reconstructing autobiographical memories involves the activation of memory networks that reflect changing retrieval processes both during covert and overt retrieval.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH R01 MH069942

Stony Brook University

Title: Thalamo-cortical coupling is task dependent during visual working memory

Authors: *A. S. HUANG, T. M. LE, H.-C. LEUNG;
Psychology, Stony Brook Univ., Stony Brook, NY

Abstract: Previous studies in nonhuman primates and rodents showed that higher-order thalamic areas play a role in modulating cortical activity during selective attention and working memory, whereas disruption of these thalamic areas can significantly influence cortical activity and produce severe cognitive deficits. Here we examined the role of the thalamus in selective visual information processing in humans. To determine whether the thalamus would exhibit enhanced coupling with the fusiform face area (FFA) and the parahippocampal place area (PPA) when face or scene stimuli respectively are behaviorally relevant, we analyzed the thalamo-cortical functional connectivity during selective maintenance using fMRI data collected from 21 healthy young adults (12 female, 9 male). Selective maintenance of either a scene (Remember Scene) or a face (Remember Face) in working memory was manipulated by a retro cue indicating which remembered item remains task relevant. The region of interest (ROI) was a part of the thalamus that showed strongest structural connectivity with the prefrontal cortex based on the literature. Psychophysiological interaction analysis (implemented using gPPI toolbox) was applied to estimate functional connectivity of the thalamic ROI. Thalamus-PPA coupling was enhanced during the delay period of the Remember Scene condition while thalamus-FFA coupling was enhanced during the delay period of the Remember Face condition. Further, greater functional connectivity between the thalamic ROI and FFA/PPA during the delay period when the preferred stimulus is task irrelevant was associated with poor discrimination of the region's activity patterns for the preferred probe stimulus. These findings can not be attributed to mean activity differences as the thalamus was about equally active on both conditions; and thalamic activity amplitude was not correlated with classification accuracy of PPA or FFA activity patterns for the face or scene probes. These findings suggest that, beyond the previously implicated prefrontal role in working memory, thalamo-cortical connectivity may also play a significant role in selective maintenance of visual information in working memory. The thalamus may be involved in prioritizing visual representations in a visual association region when that region’s preferred stimulus is task relevant.

Disclosures: **A.S. Huang:** None. **T.M. Le:** None. **H. Leung:** None.

Poster

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Title: Developmental differences in hippocampal-prefrontal mediated memory updating

Authors: *M. L. SCHLICHTING, K. F. GUARINO, A. R. PRESTON;
Univ. Texas Austin, Austin, TX

Abstract: It is uncontroversial that how we learn depends on what we already know; our knowledge stores are constantly being updated to incorporate our new experiences. In the young adult brain, related knowledge is brought to mind during new encoding and then updated through operations supported by the hippocampus and medial prefrontal cortex. Yet, how dynamic interactions between existing knowledge and new experiences shift over development remains virtually unstudied. Both the hippocampus and medial prefrontal cortex undergo continued structural change well beyond childhood, with prefrontal maturation in particular continuing into early adulthood. Previous research thus begs the question: if our underlying neural systems are maturing through middle childhood and beyond, are children and adolescents able to capitalize on their existing knowledge during new learning in the same way as adults? Here, human participants ages 7-30 years encoded AB and XY object-object associations across three study repetitions during high-resolution fMRI scanning. Critically, participants also learned related BC pairs, which included an object (B) that had been previously studied as part of an AB pair, as well as a C item from one of two visual categories (face or scene). We hypothesized that adults would preferentially reactivate related knowledge and engage updating regions during overlapping pair encoding (in the brain, condition AB ≠ XY), while children would fail to reactivate related content and use similar processes to encode all pairs, irrespective of overlap status (condition AB = XY). We found differences in hippocampal and medial prefrontal engagement across age groups specifically during overlapping (AB) encoding, suggesting developmental shifts in how we learn information that is related to prior knowledge. Using a pattern classification approach, we also found evidence for processing of the related but not but presently viewed visual category (C item, face or scene) during AB encoding in adults but not children, further suggesting that adults more readily bring their related (BC) knowledge to bear during new encoding. These results suggest fundamental shifts in the way we learn across developmental time, particularly when there is an opportunity to build upon prior knowledge. Our findings are consistent with the notion that neural maturation brings with it the ability to form increasingly flexible memories, a finding that has broad implications for how we understand and promote cognitive development in both theory and practice.

Disclosures: **M.L. Schlichting:** None. **K.F. Guarino:** None. **A.R. Preston:** None.

Poster

637. Human Cognition and Memory III

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 637.22/III13

Topic: H.02. Human Cognition and Behavior

Support: DARPA N66001-14-C-4016

Title: Decoding memory features from hippocampal CA3 and CA1 spike trains during a delayed match-to-sample task in human

Authors: *D. SONG[1], R. HAMPSON[2], B. ROBINSON[1], V. MARMARELIS[1], S. DEADWYLER[2], T. BERGER[1];
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Abstract: To understand how memory information is encoded in the hippocampus, we build classification models to decode memory features from hippocampal spiking activities recorded from human subjects performing a memory-dependent delayed match-to-sample (DMS) task. Adult patients suffering from pharmacologically refractory epilepsy are surgically implanted with FDA-approved hippocampal electrodes capable of field potential (macro-) and single-unit (micro-) recordings for localization of seizures. Single unit neural activities (i.e., spike trains) are isolated and recorded from the hippocampal CA3 and CA1 regions using a Blackrock Cervello electrophysiological recording system. Cognitive and behavioral experiments consist of visual object and spatial position oriented DMS tasks, where the patients are required to remember the sample images or the locations of sample images and retrieve these memories after a variable delay to generate correct responses. Image categories are labelled by normal human subjects to provide output signals for the classification model. CA3 and CA1 spike trains around sample responses (-2s to 2s) are taken as input signals to be classified or decoded. The classification model consists of a set of B-spline basis functions for extracting memory features from the spike patterns, and a sparse logistic regression classifier for generating binary categorical output of memory features. Classification performance are evaluated with Matthews correlation coefficients. Results from multiple human patients show that classification models can extract significant amount of memory information with respects to types of memory tasks and categories of sample images used in the task, despite the high level of variability in prediction accuracy due to the small sample size (40 - 80 trials). These results strongly support the hypothesis that memories are encoded in hippocampal spiking activities and have important implication to the development of hippocampal memory prostheses.

Disclosures: **D. Song:** None. **R. Hampson:** None. **B. Robinson:** None. **V. Marmarelis:** None. **S. Deadwyler:** None. **T. Berger:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: Templeton Foundation 36751

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Intel Corporation

Title: First you remember, then you see: Dynamic sampling from learned associations biases perceptual inference.

Authors: *A. M. BORNSTEIN[1], M. ALY[2], S. F. FENG[3], N. B. TURK-BROWNE[2], K. A. NORMAN[2], J. D. COHEN[2];
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Abstract: How does experience guide perception? Perceptual decisions are typically modeled using accumulation process that integrate sequential samples of noisy sensory input. These models have been extended to incorporate past experience via static summaries: either as an initial bias towards the most-experienced option, or as an additional weight placed on sensory evidence congruent with expectation. Here, we test the hypothesis that previous experiences can also be dynamic elements of the inference process, sequentially sampled in the same manner as perceptual input.
Fifty-six participants performed a perceptual decision task in which the likely identity of a noisy image was indicated by a pre-stimulus cue. Four fractal cues each predicted one of 4 images (2 faces, 2 scenes) with variable reliability. Critically, cue-stimulus contingencies were not instructed, but learned via experience. After experiencing the contingencies, participants performed 80 test trials, on which they viewed a cue followed by a flickering stream of images from one perceptual category; one image, the target, was more prevalent than the other. The reliability of perceptual evidence was manipulated by varying the mixture proportions of the target image and the paired same-category image. Participants pressed a response key corresponding to the predominant image in the flickering stream.
Response times were bimodal, with peaks before and after the onset of the flickering stream. This bimodality is suggestive of two noisy accumulation processes: one relying on samples from learned expectations and beginning at cue onset, and the other relying on observed perceptual input and beginning at the onset of the flickering stream. Regression analyses and a formal model comparison, using a multi-stage drift-diffusion model, confirmed that behavior was

indeed best described by two accumulation processes with different drift rates, with the second process initializing at the ending state of the first.
Multivariate pattern analyses in an fMRI experiment (n=32) were used to measure neural evidence for the category of the upcoming images in the flickering stream, after cue onset but before the images appeared. Fluctuations in neural category evidence during this anticipatory period were a significant predictor of RTs, even after accounting for the other modeled factors. Together, these results show that learned expectations are a dynamic source of evidence during perceptual inference, and suggest that neural reactivation of anticipated percepts is the mechanism by which this occurs.

Disclosures: **A.M. Bornstein:** None. **M. Aly:** None. **S.F. Feng:** None. **N.B. Turk-Browne:** None. **K.A. Norman:** None. **J.D. Cohen:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant MH100121

Title: Medial prefrontal cortex supports retrieval of integrated memories

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Abstract: Memories are not formed in isolation, but rather are learned in the context of a vast store of existing knowledge. In particular, medial prefrontal cortex (PFC), through its interactions with hippocampus, has been implicated in a memory integration process whereby existing memories are updated with new information. Recent data indicate medial PFC engagement during encoding of content that overlaps with existing knowledge promotes inference decisions that require participants to reason about the relationships among indirectly experienced events. Here, we test the idea that medial PFC would be engaged selectively for inference judgments that rely on the retrieval of integrated representations versus those that rely on retrieval and recombination of individual memory traces. Specifically, we used multivariate pattern classification to quantify reactivation of existing memories during encoding of overlapping associations, which has previously been shown to be an index of memory integration. We hypothesized that evidence for encoding-related integration would be associated with enhanced engagement of medial PFC during inference itself. To test this hypothesis,

participants first learned face-object and scene-object (denoted AB) pairs. During fMRI scanning, participants then studied overlapping associations (BC) for which one object (the B item) was shared with a previously studied paired. This encoding phase was followed by a scanned inference task, during which participants made judgments about the indirect relationships between A and C items. Using multivariate pattern analysis, we isolated regions that support inference through integration by relating brain activation during correct inference trials separated by the degree of memory reactivation (A faces and scenes) during overlapping event (BC) encoding. We found that the degree to which participants reactivated related memories (A items) during encoding of overlapping (BC) associations predicted individual differences in reaction time for correct inference: Faster reaction times were associated with greater evidence for encoding-based integration. Furthermore, we found that medial PFC was selectively recruited during correct inference trials for which there was greater memory reactivation during encoding of the corresponding overlapping association. This finding is consistent with the prediction that medial PFC biases retrieval to access integrated memory networks and suggests that medial PFC serves an important role in organizing memories for related events.

Disclosures: N.W. Morton: None. **A.R. Preston:** None.

Poster

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Support: John Templeton Foundation

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Title: Complementary learning systems within the hippocampus: A neural network modeling approach to reconciling episodic memory with statistical learning

Authors: *A. C. SCHAPIRO[1,2], N. B. TURK-BROWNE[2], M. M. BOTVINICK[3], K. A. NORMAN[2];
[1]Psychiatry, Beth Israel Deaconess Med. Ctr. / Harvard Med., Boston, MA; [2]Dept. of Psychology and Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [3]Google DeepMind, London, United Kingdom

Abstract: The complementary learning systems (CLS) theory (McClelland, McNaughton, & O'Reilly, 1995, *Psychol Rev*) highlighted the computational tradeoff between tracking the details of individual experiences (episodic memory) and the regularities shared across these experiences (statistical learning), and posited that these two processes must be supported by different brain systems. In particular, the hippocampus uses a high learning rate and pattern separated representations to encode individual experiences, whereas the cortex benefits from a slow learning rate and overlapping representations in extracting regularities. However, this division of labor conflicts with recent empirical findings that the hippocampus is involved in statistical learning (Schapiro et al., 2012, *Curr Biol*; 2014, *JOCN*; 2016, *Hippocampus*). To reconcile the theory with these data, we ran simulations of statistical learning tasks using a hippocampal neural network model that instantiates the episodic learning component of the CLS theory (Ketz et al., 2013, *PLOS Comp Biol*). The model contains hippocampal subfields dentate gyrus (DG), CA3, and CA1, which learn to map between input in superficial layers of entorhinal cortex (EC) to output in deep layers of EC. There are two main pathways: The trisynaptic pathway (TSP), EC -> DG -> CA3 -> CA1, and the monosynaptic pathway (MSP), EC <-> CA1. The DG and CA3 subfields employ sparse connectivity and high inhibition, making the TSP the engine for pattern separation of episodic memories in the hippocampus. CA1 employs more overlapping representations and a relatively slower learning rate — it is more cortex-like — and has acted in extant models as a translator between sparse TSP representations and overlapping EC representations. When we exposed the model to input with temporal regularities, the MSP/CA1 took on a new role: it represented the temporal regularities, allowing the model to do rapid statistical learning. We will present simulations that demonstrate: (i) the complementary roles of the TSP and MSP in episodic and statistical learning, respectively; (ii) temporally predictive behavior and representations; (iii) preserved statistical learning in simulations of infants, who have underdeveloped TSPs; and, (iv) higher-order learning in temporal community structure and associative inference paradigms. Our simulations suggest that there are complementary learning systems within the hippocampus itself, the TSP and the MSP, which allow it to support both rapid episodic and statistical learning.

Disclosures: A.C. Schapiro: None. **N.B. Turk-Browne:** None. **M.M. Botvinick:** None. **K.A. Norman:** None.

Poster

637. Human Cognition and Memory III

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Topic: H.02. Human Cognition and Behavior

Support: Templeton foundation

Title: Better in sequence: The role of task similarity in encoding and executing planned task sequences

Authors: *I. MOMENNEJAD[1], C. REVERBERI[2], S. MUSSLICK[1], J. COHEN[1], J.-D. HAYNES[3];
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[3]Bernstein Ctr. for Computat. Neurosci., Humboldt Univ. Berlin, Berlin, Germany

Abstract: Most plans include orderly sequences of multiple tasks that vary in similarity. Successful realization of task sequences requires the brain to encode all tasks simultaneously, and retrieve and execute them in the correct order. However, it remains unclear how the similarity of the tasks in a planned sequence affects their computational encoding and behavioral performance, and how similarity effects change over time. Here we employed a behavioral study, functional magnetic resonance imaging (fMRI), and computational modeling in order to address these questions. On each trial participants were instructed to either perform (a) a single task or (b) a sequence of two tasks. The tasks could be parity, magnitude, or color judgment on that trial's stimulus: a number in red or green font. Notably, the numerical tasks (parity and magnitude) were more similar to each other than to color judgment. Thus, two of six sequences had similar tasks (similar sequences) and four of six dissimilar (dissimilar sequences). We found a "similarity benefit": reaction times to a given task in a similar sequence were faster compared to performing it alone. This similarity benefit was larger for the second task. We found a "dissimilarity cost" for the first task: the same task was slower as the first in a dissimilar sequence than as a single task, and the benefit of the second task was diminished in dissimilar sequences. Comparing the first run (48 trials) to the last, we found that (a) both similarity benefits and dissimilarity costs diminished over time and (b) overall there was a cost to going first and a benefit to going second. To study the encoding of task representations, we trained a searchlight multi-voxel pattern classifier on brain volumes collected during the preparation period of "single task" trials. Using this classifier, we were able to decode the identity of the upcoming tasks in "sequence trials", from frontoparietal regions (rostrolateral PFC and posterior parietal cortex) of volumes collected during the preparation period. With representational similarity analysis (RSA), we investigate whether the observed behavioral changes over time correspond to changes in the similarity of task representations in the brain. Furthermore, we are developing a neural network model of task representations to investigate mechanistic explanations of (a) when performing a task in a sequence has benefits, or costs, over performing it alone, and (b) how changes in task representations over time lead to changes in the behavioral costs and benefits of sequences. These findings bear novel consequences for a mechanistic understanding and optimal planning of task sequences.

Disclosures: I. Momennejad: None. **C. Reverberi:** None. **S. Musslick:** None. **J. Cohen:** None. **J. Haynes:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

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NSF GRFP

Title: Time-dependent restructuring of memory representations strengthens associations between overlapping memories

Authors: *A. TOMPARY[1], L. DAVACHI[2];
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Abstract: Semantic memories are thought to emerge from the accumulation of commonalities across discrete episodic events. However, our understanding of the neural mechanisms underlying this transformation is limited. Specifically, it is unclear how memories with overlapping components are represented in terms of their shared and distinct features over time and how this supports behavior. We developed a two-day fMRI study to ask how memories become represented according to their shared or distinct components over time, and how these representations influence behavior. Each day, subjects incidentally learned a different set of objects presented in a continuous sequence. Unbeknownst to the subjects, the sequences were arranged such that some objects had an overlapping temporal context of two items. Specifically, these objects were always preceded by an identical sequence of two objects. Immediately after the second encoding session, participants completed a recognition memory test with objects from both encoding sessions. Trials were arranged to test for the integration of objects with overlapping temporal contexts. Recognition was faster when an object with an overlapping context primed the tested object. Importantly, this effect emerged only for objects learned 24 hours prior to the test. This suggests memories with overlapping information become associated over time. Furthermore, when focusing on the remotely learned sequences, participants with a stronger priming effect exhibited a greater increase in similarity between the objects that were studied with overlapping temporal contexts. Critically, no such relationship existed for the recently learned sequences. Planned fMRI analyses will assess the relationship between time-dependent changes in object representations and computations supporting the encoding of the sequences.

Disclosures: **A. Tompary:** None. **L. Davachi:** None.

Poster

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Title: Critical moments of learning are mediated by distinct hippocampal and frontoparietal encoding processes

Authors: *M. L. MACK[1], A. R. PRESTON[1], B. C. LOVE[2,3];
[1]The Univ. of Texas At Austin, Austin, TX; [2]Univ. Col. London, London, United Kingdom; [3]The Alan Turing Inst., London, United Kingdom

Abstract: Successful learning depends on encoding newly-learned information relative to existing knowledge. Neurobiological memory theories attribute such encoding to complementary functions of the hippocampus (HPC) and its interactions with prefrontal cortex (PFC). Specifically, HPC comparator processes that evaluate the overlap between new experiences and current knowledge trigger either pattern separation, wherein new memories are made representationally distinct from existing knowledge, or pattern integration processes mediated by PFC, wherein new information is incorporated into existing memories. Recent electrophysiological data suggest that the tendency of the HPC to trigger separation or integration may fluctuate on a moment-by-moment basis; yet, characterizing such moments of learning have proved challenging in humans given the lack of empirical methods to identify precisely when knowledge is created versus updated. Here, we overcome this challenge with a computational learning model that makes explicit predictions about when newly-encountered information is encoded through pattern separation or pattern integration. During fMRI scanning, participants learned to categorize complex objects composed of multiple features across four category learning tasks, with each task defined by different associations between category labels and combinations of object features. The learning model was fit to participants' behavior during the four learning tasks to derive trial-by-trial predictions of when a new separate memory was formed versus when existing knowledge stores were updated. These model predictions were then leveraged in univariate and multivariate classification analyses to distinguish the neural circuits

underlying moments of knowledge creation and integration. Whereas HPC was associated with model predictions for both knowledge creation and integration, separable frontoparietal regions were distinctly associated with the different encoding operations. We found a knowledge creation network associated with medial PFC and angular gyrus, and a knowledge integration circuit comprised of frontopolar, dorsolateral prefrontal, and superior parietal cortices. Our findings establish a neurocomputational framework grounded in cognitive theory for investigating how the interaction of new experiences and existing knowledge mediate successful learning.

Disclosures: **M.L. Mack:** None. **A.R. Preston:** None. **B.C. Love:** None.

Poster

637. Human Cognition and Memory III

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Topic: H.02. Human Cognition and Behavior

Title: Retroactive and graded modulation of memory by reward

Authors: *E. K. BRAUN[1], G. WIMMER[3], B. VAIL[2], C. VAN GEEN[2], D. SHOHAMY[2]; [1]Psychology, [2]Columbia Univ., New York, NY; [3]Stanford Univ., Palo Alto, CA

Abstract: Adaptive decision-making can be supported by a model of how sequences of events lead to rewards. Thus, it is essential not only to remember an event that was rewarding, but also the series of events that led to it; however, often we do not know which events will later turn out to be important. To solve this problem, it is necessary for rewards to retroactively prioritize neutral memories based on later outcomes. Here we addressed the question of whether and how such retroactive modulation enhances memory for sequences of events leading to reward. Our predictions were based on two distinct physiological processes in the hippocampus. First, electrophysiological recordings in rats show that after running through a maze to a reward, hippocampal place cells replay recent experiences in reverse, which may provide a mechanism by which dopaminergic reward signals are propagated back to sequences of previous experiences. Second, dopamine increases cellular plasticity in the hippocampus. Together, these two processes provide a putative mechanism by which rewards could retroactively enhance memory. We predicted that reward would have a graded effect on memory for sequences of neutral events that preceded it, with the greatest enhancement for events that were most proximal to the reward. To test this, we developed a task in which participants navigated through a series of grid mazes, one space at a time, searching for a hidden gold coin worth $1. In each space, an incidental, trial-unique object was presented before participants moved to the next space. We

manipulated the maze outcomes so that half of the mazes ended in reward. Each maze was followed by a short rest break (15-25s). We administered a surprise recognition memory test either 24-hours or 15-minutes after encoding, allowing us to measure the effect of consolidation on reward-enhanced memory. We found that participants' memory for the objects was retroactively modulated by reward, such that memory was selectively enhanced for objects closest to the reward. This interaction between reward and proximity on memory was selective to the 24-hour condition and not found in the 15-minute condition. Moreover, we found that the reward-proximity interaction became pronounced as the length of the post-maze rest block increased. These findings demonstrate that reward retroactively enhances episodic memory for preceding neutral events, modulating memory as a function of it sequential proximity to reward. This effect is selective to reward, requires consolidation, and varies as a function of the rest time after encoding, consistent with neurobiological models of how the brain integrates memories across time and space.

Disclosures: E.K. Braun: None. **G. Wimmer:** None. **B. Vail:** None. **C. van Geen:** None. **D. Shohamy:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01EY021717

NIH Grant F32EY024851

Title: The influence of recent semantic learning on human visual cortex

Authors: *M. N. COUTANCHE[1], S. L. THOMPSON-SCHILL[2];
[1]Dept. of Psychology, Univ. of Pittsburgh, Pittsburgh, PA; [2]Dept. of Psychology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Humans are frequently exposed to information about the many objects, animals and people we that encounter in our environments. In addition to a suite of perceptual processes that can extract information from observed items, humans have the unique ability to discover the properties of objects through abstract communication. We have investigated how the introduction of visually relevant information -the real-world size of recently introduced animals- impacts the patterns of neural activity observed in a person's visual cortex. We scanned human

participants with functional Magnetic Resonance Imaging (fMRI) while we introduced them to novel and known animals and tools. At the start of the experiment, participants were exposed to images of these items through a simple 1-back task. Participants were next presented with, and tested on, new factual information about each new concept. Finally, a post-learning 1-back task was administered. We used multivariate pattern analysis (MVPA) to decode the neural patterns underlying the processing of the new and known animals and tools, in order to compare activity patterns before and after learning. We find that after introducing people to new information about items' real-world size, visual activity patterns for the newly introduced animals more closely resemble the activity patterns observed for similarly sized known animals. In contrast, learning semantic information about the intended motor manipulation of a new tool did not affect future visually generated activity patterns. Our findings suggest that learning information about a new visual concept might rapidly influence visual cortex neural activity.

Disclosures: M.N. Coutanche: None. **S.L. Thompson-Schill:** None.

Poster

638. Learning and Memory: Rodent Studies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 638.01/III22

Topic: H.01. Animal Cognition and Behavior

Title: The effect of diet exposure on impulsive choice in rats: delay and reward sensitivity

Authors: *C. C. HILL, J. R. A. PIRKLE, K. KIRKPATRICK;
Kansas State Univ., Manhattan, KS

Abstract: Recent research suggests that there is a correlation between diet and impulsivity; specifically, those who consume a diet high in fat and sugar tend to be more impulsive. However, human research is unable to disentangle the direction of this relationship. Recent research from our laboratory suggests that high-fat and high-sugar diets can cause greater impulsive choice behavior. Given that diet can induce impulsivity and potentially explain the relationship between impulsivity and obesity in humans, it is critical to determine how the effects of diet on impulsivity can be addressed. One key aspect to improving impulsive choice is understanding the mechanisms of impulsive choice: delay and reward sensitivity. The present study assessed diet effects on delay and reward sensitivity and the implications for impulsive choice behavior. Rats were divided into three groups (high-fat, high-sugar, and control) where diet composition differed, and each group was given access to the same number of calories to each day. The high-fat group received chow with a lard supplement, the high-sugar group received chow with powdered sugar icing, and the control group received all chow. After 8-

weeks of exposure to the diet, all rats were then tested on two impulsive choice tasks in which individuals were presented with a choice between a smaller reward available sooner (SS) or a larger reward available later (LL). One impulsive choice task manipulated the delay to the SS choice and the other task manipulated the magnitude of the LL reward. After the impulsive choice tasks, rats completed a bisection task to determine timing deficits and a reward sensitivity task to determine their ability to discriminate between different reward amounts. The results from this experiment replicated the previous study from our lab showing that a diet high in fat or sugar induces impulsive choice, which may explain the relationship between impulsivity and obesity. In addition, it outlines the deficits of impulsive choice that could be targeted with a behavioral intervention to improve impulsive behavior that results from poor diet consumption.

Disclosures: **C.C. Hill:** None. **J.R.A. Pirkle:** None. **K. Kirkpatrick:** None.

Poster

638. Learning and Memory: Rodent Studies

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Program#/Poster#: 638.02/III23

Topic: H.01. Animal Cognition and Behavior

Support: R01-MH-085739

Title: The durability and generalizability of neurocognitive intervention effects on impulsive choice in rats

Authors: *A. T. MARSHALL, J. R. PETERSON, C. TURPEN, K. KIRKPATRICK;
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Abstract: Impulsive choice behavior refers to the preference for a smaller-sooner (SS) reward over a larger-later (LL) reward when choosing the LL reward is optimal in terms of reward rate. Greater impulsive choice has been observed in individuals with ADHD, schizophrenia, and depression, as well as in substance abuse disorder, obesity, and pathological gambling. Accordingly, there have been recent efforts to develop neurocognitive interventions that may be implemented to alleviate maladaptive impulsive choice. Given the delays involved in waiting for reward in impulsive choice tasks, many interventions have targeted individuals' abilities to anticipate and time the delivery of upcoming reward, with the goal of reducing aversion to long reward delays. These efforts have been successful, suggesting that the interventions do improve self-control immediately after exposure to these interventions. However, the durability and generalizability of these neurocognitive interventions have yet to be thoroughly tested. The goal of these experiments was to investigate whether time-based neurocognitive interventions produce

long lasting (Experiment 1) and generalizable effects across different decision-making parameters (Experiment 2). In Experiments 1-2, three groups of rats were exposed to one of two intervention tasks (fixed or variable delays to reward) or a control task (i.e., no delay to reward); the fixed and variable delays were in the range of delays that were experienced in the impulsive choice task. In the subsequent impulsive choice task, SS choices resulted in 1 food pellet after 5, 10, and 20 s in successive phases, while LL choices always resulted in 2 food pellets after 30 s. Here, the time-based intervention groups made fewer impulsive choices than the control group. In Experiment 1, the rats were then exposed to this same paradigm approximately 6 months later; the rats remained in their home cages during the waiting period. In Experiment 2, the rats were then exposed to different choice paradigms, in which reward magnitude or reward delay was manipulated. Exposure to both time-based interventions reduced impulsive choice behavior across the subsequent testing of impulsive choice. Overall, these results lend further support to the use of non-invasive neurocognitive interventions to reduce impulsive choice in rats, thus unveiling a critical preclinical avenue that may be navigated for future implementation in reducing impulsivity in individuals in various subpopulations prone to making impulsive decisions.

Disclosures: **A.T. Marshall:** None. **J.R. Peterson:** None. **C. Turpen:** None. **K. Kirkpatrick:** None.

Poster

638. Learning and Memory: Rodent Studies

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Topic: H.01. Animal Cognition and Behavior

Support: NIH T32

Title: A novel open-field task for the analysis of episodic-like memory and its substituents

Authors: ***S. STUEBING**, K. KIRKPATRICK;
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Abstract: Episodic memory is one of the first memory types to be compromised in both aging and dementia. One unique feature that distinguishes episodic memory from other memory types is its composition of three substituents - object recognition and discrimination (what), spatial navigation (where), and timing (when), which must be appropriately integrated for episodic encoding and recall. Research on episodic-like memory, the animal counterpart thereof, highlights the three underlying components of the memory type by challenging animals to

successfully assimilate previously learned what, where, and when information to solve a task. This provides an avenue for studying the memory type as a whole, as well as individual differences that exist within the what, where, and when components, which could affect an individual's ability to utilize episodic-like memory. However, current episodic-like tasks do not directly assess the what, where, and when components, nor do they evaluate whether individual differences within the substituents affect an individual's ability to successfully perform the integrated episodic-like task. But, better assessment of the substituents may provide novel insight into the nature of episodic memory decline, and therefore merits further research. As such, this project aimed to develop a novel task for studying both episodic-like memory and its substituents in rodents. By creating and piloting an open-field task that utilizes scent (what), location (where), and time of day (when), this task successfully elicited the use of episodic-like memory in rats, and elucidated individual differences in the what, where, and when substituents of episodic memory. These findings indicate that rats exhibit individual differences in the underlying what, where, and when components of episodic memory, a more thorough understanding of which could provide further insight into the nature and eventual prevention of the episodic memory decline that is seen in both aging and dementia.

Disclosures: S. Stuebing: None. **K. Kirkpatrick:** None.

Poster

638. Learning and Memory: Rodent Studies

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JSPS KAKENHI 11J06508

Title: Systemic injection of clonidine, an alpha2-adrenergic auto-receptor agonist, interferes with state dependent modulation of hippocampal theta-gamma coupling during a spatial decision-making task

Authors: *S. AMEMIYA, A. D. REDISH;
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Abstract: The hippocampal theta oscillation (6-12 Hz) is thought to play a role in deliberative processes in decision making, interacting with gamma oscillations [low gamma (LG): 30-55 Hz, high gamma (HG): 60-90 Hz]. However, how the interaction of theta and gamma oscillations

modulates the decision process is poorly understood. Previous studies have reported that the noradrenergic drug clonidine makes animals more decisive, reflected as a decrease in deliberation-related head re-orienting behavior of rats (vicarious trial-and-error [VTE]), and a decrease in the alternation of hippocampal representations of possible options at a choice point. In order to test how theta and gamma oscillations underlie deliberative processes, we examined the influence of clonidine on hippocampal theta and gamma oscillatory activity from rats running a decision-making task.
Rats ran a modified Hebb-Williams maze, consisting of a changeable central path, a final decision point, and rewarded return rails leading to the start of the loop. On each lap, only one side or the other was rewarded. Three reward-contingencies were used: turn left, turn right, or alternate for reward. During the analyzed probe trials, the rewarded rule changed approximately halfway through the session. On a subset of trials, clonidine (30 μg/kg) or saline vehicle was delivered IP 30 min before the run. Hippocampal local field potentials (LFP) and cell spiking activity were recorded from the hippocampal CA1 and fissure regions. Theta phase was calculated based on actual waveform of theta. The peaks and troughs of theta waves were detected and assigned as phase 0, 180, respectively. Furthermore, we analyzed the asymmetry of each theta wave as the ratio of the length of the descending part (peak to trough) and the ascending part (trough to peak).
The theta wave was asymmetric, with the descending part longer than the ascending part. Consistent with previous studies, gamma oscillations appeared at particular phases of theta: LG power increased in the ascending phase and HG power increased in the descending phase. More symmetric theta waves included more LG power. Activity of place cells indicated that the ascending part of theta included more prospective information than the descending part. At the final decision point, control sessions showed an increase of power in LG and HG on VTE laps compared to non-VTE laps. In addition, the theta wave was more symmetric on VTE laps than on non-VTE laps around the choice point. These changes were suppressed by clonidine. Our findings suggest that hippocampal theta-gamma coupling reflects deliberative processes which can be modulated by noradrenaline manipulations.

Disclosures: **S. Amemiya:** None. **A.D. Redish:** None.

Poster

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Title: Using a novel neuroeconomic decision-making foraging task to test mouse models of addiction

Authors: *B. SWEIS[1], A. J. ASP[2], C. ZHENG[2], S. R. K. BRANCEL[2], M. J. THOMAS[2], A. D. REDISH[2];
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Abstract: New theories see addiction as a result of failures of decision-making processes. To test how cocaine exposure influenced economic decision-making, we adapted a novel foraging task for mice (Restaurant Row). The task consisted of 4 reward zones (restaurants) around a square, which were primed serially, forcing mice to run around the square, foraging for food. We modified the task to include a separate *decision zone* for each restaurant. Upon entry into a decision zone for a given restaurant, an offered delay was signaled by a repeating tone (pitch indicated delay). The mouse could then either enter the restaurant, at which time the delay started counting down (indicated by descending pitch of the delivered tones) or leave the decision zone (skipping the offer). Mice also had the opportunity to quit waiting mid-countdown. Each restaurant provided a uniquely flavored food reward. Delays were random at each encounter (1-30s) and mice had 1h to get their food for the day. This means they had a budget of time to spend by making stay or skip decisions to each encountered offer.
After 30d of task training, 8 C57BL/6J male mice received 5d of cocaine or saline (15mg/kg IP) while being tested on RRow daily. RRow testing took place during the day with injections in the evening.
Thresholds of willingness to wait were determined by fitting sigmoid curves to (1) skip/enter decisions and (2) quit/earn decisions as a function of offer length. Mice demonstrated stable skip/enter and quit/earn thresholds for different flavors revealing individual preferences similar to both rats and humans on these foraging tasks. Cocaine increased quit/earn thresholds but not skip/enter thresholds. Mice spent varied time in the decision zone, but took longer to skip offers than to accept them. When mice accepted offers that they later quit, they spent more time in the decision zone before accepting those offers than they did when accepting offers and waiting through the entire countdown. In other words, decisions to stay that resulted in earned pellets were made the quickest. Cocaine exposure increased time to accept offers but did not affect time to skip. Cocaine mice spent the most time deciding to accept offers that they later ended up quitting - behavior that is economically unfavorable. Mice displayed vicarious trial and error (VTE) behaviors in the decision zones, and showed more VTE when deciding to skip, suggesting

mice were deliberating more on these occasions. Cocaine mice displayed less VTE on these skip trials as well as on enter-then-quit trials, suggesting that cocaine exposure promotes decision-making behavior that is suboptimal on an economic task, possibly by reducing an overall capacity to deliberate.

Disclosures: **B. Sweis:** None. **A.J. Asp:** None. **C. Zheng:** None. **S.R.K. Brancel:** None. **M.J. Thomas:** None. **A.D. Redish:** None.

Poster

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SFN Neuroscience Scholars Program

Title: DREADDs disruption of prelimbic cortex alters hippocampal SWR dynamics during rest after a foraging task in rats

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Abstract: Hippocampal (HC) place cell assemblies “replay” spatial trajectories previously traversed in a temporally condensed manner during sharp wave/ripple complexes (SWR, 150-200 Hz) at food-reward sites on mazes and during post-run rest. Recent experiments have suggested an important coupling of HC and prelimbic (PL) cortex during decision-making. Recently, PL has been implicated in the retrieval and selection of hippocampal memory representations, biasing the retrieval of goal-related representations. However, a role for PL in HC replay consolidation remains largely unexplored.
We examined how PL affects HC SWR activity by reversibly disrupting PL in rats with virally-delivered h4MDi DREADDs, while recording neural ensembles from HC, PL, and ventral striatum (vStr) simultaneously from rats trained on the spatial neuroeconomic task Restaurant Row. This task reveals individual preferences of reward flavor and the cost the rat was willing to

exert to receive it. Rats ran around a circular maze divided into four zones with four evenly spaced spokes. One of four different food rewards (fruit, banana, chocolate, unflavored) was delivered at the end of each spoke. On entry into each zone, a tone indicated the delay the rat would have to wait for food and the rat was able to choose to stay (waiting out the delay) or skip the offer and move on to the next option.
Rats were run for 20 days each on this task, with alternating dosages of CNO (5 mg/kg, subQ) and vehicle control. Behavioral results from these manipulations has been previously reported, but in short, PL reduced the thresholds and disrupted vicarious trial and error behaviors. Neurophysiologically, systemic injections of CNO altered local and global neural dynamics in PL DREADD-transduced rats. CNO reduced cell yield in PL without affecting HC or vStr cell yields. CNO reduced the mutual information between spiking and task components in PL, but not in HC or vStr. CNO decreased theta (6-10 Hz), low gamma (25-55 Hz) and high gamma (65-115 Hz) power in PL. Further, CNO altered network dynamics in HC including decreasing theta and low gamma power. Interestingly, disrupting PL dynamics resulted in altered neural dynamics during HC replay. CNO administration reduced the number of HC SWR events seen during post-run rest and altered the distribution between forward and backward replays. Further, CNO in PL DREADD-transduced rats altered LFP dynamics between HC and PL as well as the HC and vStr, but not between PL and vStr, by significantly altering power correlations between low gamma and SWR frequencies.

Disclosures: **B. Schmidt:** None. **A.D. Redish:** None.

Poster

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CLA Brain Imaging Project Grant from the University of Minnesota

Title: The neural correlates of experiential foraging in humans

Authors: *S. V. ABRAM[1], A. D. REDISH[2], A. W. MACDONALD, III[1];
[1]Psychology, [2]Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Experiential foraging requires that animals economically allocate scare resources such as time. Animal models suggest that these allocations occur through interacting decision-making processes. To further develop the translation between rodent and human foraging models, we employed an experiential stay/go foraging task (the "Web-Surf Task," akin to the "Restaurant Row" rodent task). Human subjects (current N=9) had 40 minutes to forage for 4s videos from one of four categories (kittens, dance, landscape, bike-accidents), encountered sequentially. Videos were used because they are consumable on a trial-by-trial basis. Subjects were informed of the delay before video presentation. They then chose to stay and wait for the video, or skip to the next category. Viewed videos were rated on a 1-4 scale. To travel between categories, subjects clicked the numbers 1-4 as they randomly appeared on the screen.
Neurophysiological findings from rodents on the Restaurant Row task suggest that the orbitofrontal cortex (OFC) and ventral striatum are likely involved in expectations of reward, and the hippocampus (HC) may be implicated in episodic future thinking. To further develop the neurophysiological translation between species, human subjects completed the Web-Surf task during 3T functional imaging (45'; TR = 720ms; $2mm^3$).
Behavior: Humans showed individual preferences for each video category, revealing their preferences through thresholds (below which they would stay, above which they would skip). Delay thresholds correlated with stated preferences (video ratings, post-test category rankings), finding that 78% of correlations were above 0.75 for both ratings and rankings.
Neuroimaging analyses: Group-level models with choice (decision), delay (anticipation), and video viewing (consumption) events revealed anterior cingulate cortex (ACC) activation during decision, and ACC, anterior insula (AI)/OFC, HC, and amygdala activation during consumption ($p < 0.05$); all three events activated visual cortex. A composite map of decision > anticipation and consumption > anticipation found overlapping activation in the ACC, AI, HC, and visual cortex. Lastly, a contrast of stay > go (during decision) revealed activation in visual cortex. These data suggest that humans are imagining the reward when deciding, perhaps more so when electing to stay and wait. Consistent with human imaging findings, the salience network (i.e., ACC and AI) were engaged when evaluating primary rewards (e.g., videos). HC activation during decision-making is also in line with human and rodent research that links this area with imagination and episodic future thinking.

Disclosures: S.V. Abram: None. **A.D. Redish:** None. **A.W. MacDonald:** None.

Poster

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Title: A two-step decision-task for rats reveals behavioral correlates of model-based and model-free decisions

Authors: B. M. HASZ[1], *A. D. REDISH[2];
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Abstract: Current theories suggest that mammalian decision-making depends on separable systems, including deliberation (simulation and evaluation of potential outcomes, a "model-based" mechanism) and procedural (recognition of a situation and release of well-learned action-chains, a "model-free" mechanism). Theories suggest that rat behaviors (vicarious trial and error (VTE) and path-stereotypy) reveal the use of these systems. VTE is a behavior occasionally displayed by rats as they come to a choice point in a maze and look back and forth between potential paths, and is thought to reflect an internal deliberative process. In contrast, path-stereotypy describes how a rat's behavior becomes more regular with experience. Stereotyped paths through a maze have been hypothesized to indicate procedural behavior.
A new two-step decision task dissociates model-based and model-free choices in humans (Daw et al., 2011, Neuron). We adapted this task for rats to evaluate how these rodent behavioral signatures correlate to model-based and model-free choice. The task consists of a spatial loop, in which rats encounter two left-right choices. The second choice occurs in the presence of three computer monitors, which enable dynamic control of context. The first choice (C1: A/B) probabilistically determines the context of the second choice (C2: C/D or C3: E/F). On selecting the second choice, the rat encounters a delayed reward. The four delay times (C, D, E, and F) randomly walked slowly throughout the experiment, so the rat was constantly trying to find its way to the context and choice with the shortest delay.
7 rats were trained until they were running reliably and then run on this task for 50d each. We fit several algorithms to each rat's choices: (1) random, (2) model-based, (3) model-free, (4) a constant-weight mixed algorithm, and algorithms in which (5) VTE at C1 indicated model-based decision-making, (6) VTE at C2/C3 indicated model-based decision-making, and (7) path-

stereotypy of the lap indicated model-free decision making. Consistent with the human data, the constant-weight mixed algorithm fit the data better than either the model-based or model-free algorithms alone, all of which fit better than random. However, we found that algorithms where the model-based influence depended on the amount of VTE and the model-free influence on the degree of path stereotypy fit rat behavior better than the constant-weight algorithm. This suggests that multiple decision systems do not contribute statically to choice, but rather that the influence of each system changes over time, with the amount of influence of each system revealed by behavioral correlates such as VTE and path stereotypy.

Disclosures: B.M. Hasz: None. **A.D. Redish:** None.

Poster

638. Learning and Memory: Rodent Studies

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Topic: H.01. Animal Cognition and Behavior

Title: Effects of blast-induced mild traumatic brain injury on cognitive flexibility in rats

Authors: *C. C. TENN[1], N. CADDY[2], M. GARRETT[2], A. GIVENS[3], B. POPESCU[3]; [2]Casualty Mgmt. Section, [1]DRDC Suffield Res. Ctr., Medicine Hat, AB, Canada; [3]Dept of Anat. and Cell Biol., Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: Introduction: Traumatic brain injury (TBI) can result in a range of impairments from mild to severe and is an increasing concern for individuals in sports and the military. For military personnel, encounters with blast explosives frequently result in TBIs in particular, the mild form of this brain injury. Mild TBI can result in disturbance of cognitive, behavioural, emotional and physical functions. Several brain regions are commonly affected with this type of injury including the pre-frontal cortex that is responsible for the regulation of executive functioning. There are limited studies examining complex cognitive deficits such as decrease executive functions in experimental models of TBI. This study used an attentional set-shifting task (AST) to evaluate the effect of blast-induced mild brain injury on executive function and behavioural flexibility performance in rats. **Methods:** Rats were housed under controlled environmental conditions of light (12h light/dark cycles) and temperature. A mild brain injury was induced in anaesthetized animals by exposure to a single shockwave intensity of 140 kPa for 7.1ms duration. Control animals underwent the same treatment except for the shockwave exposure. Animals were placed on a food restricted diet for 7 days prior to blast exposure. Following the exposure, both groups of animals were trained and tested on the AST which involved a series of discriminative tasks of increasing difficulties. Four days post-exposure, brain tissues from these

animals were harvested for histopathological analyses. **Results:** There was no significant difference in body weights between the two groups before or after the blast exposure. All animals successfully completed the training phase reaching criterion for the simple discrimination trials. Blast animals required significantly more trials to reverse a learned rule. These animals also took significantly more trials to criterion to learn a new rule in another attentional dimension (extradimensional shift) as compared to controls. Brain sections stained with hematoxylin and eosin and luxol fast blue did not show any significant histopathological differences between the two groups at this early time point. **Conclusion:** These findings demonstrate early deficits in executive function and behavioural flexibility following a mild brain injury. Future studies will examine the timeline for cognitive recovery as well as the use of other histological methods (i.e. immunohistochemical staining procedures) for subtle changes in this type of mild brain injury.

Disclosures: C.C. Tenn: None. **N. Caddy:** None. **M. Garrett:** None. **A. Givens:** None. **B. Popescu:** None.

Poster

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Title: (R)-fluoxetine is more effective than (S)-fluoxetine in enhancing learning and cognitive flexibility in mice

Authors: *S. MARWARI[1,2], G. S. DAWE[3,2];
[2]Drug Develop. Unit, Life Sci. Inst., [3]Pharmacol., [1]Natl. Univ. of Singapore, Singapore, Singapore

Abstract: Fluoxetine, a clinically successful selective serotonin reuptake inhibitor, is a racemic mixture of (R) and (S) stereoisomers. In preclinical studies, chronic fluoxetine treatment (10 mg/kg) has been shown to have antidepressant effects in behavioral paradigms that has been correlated with increased adult hippocampal neurogenesis. However, the clinical evidence on whether or not fluoxetine treatment directly brings about cognitive enhancements is still under investigation and the contribution of the enantiomers of fluoxetine in these paradigms remains

largely unknown. In this study, we investigated the effects of (R) and (S)- fluoxetine treatment on antidepressant and cognitive behavioural paradigms, and cell proliferation in the hippocampus of C57BL/6J female mice. (R)- fluoxetine had superior effects over (S)- fluoxetine in elevated plus maze (p <0.001), forced swim test (p <0.05) and tail suspension (p <0.05) tests. Likewise, in a behavioural sequencing task in the IntelliCage, in which discriminated spatial patterns of rewarded and never rewarded corners were serially reversed, (R)- fluoxetine treated mice showed rapid acquisition of the behavioural sequencing (compared to the S-fluoxetine) and cognitive flexibility in the subsequent reversal stages both in the intra- and inter-session analyses (p <0.0001). (R)-fluoxetine administration also increased neurogenesis in the hippocampus, in particular in the suprapyramidal blade of the dentate gyrus, which has been reported to play a crucial role in supporting spatial learning. The results suggest that (R)-fluoxetine that is reported to have a shorter half-life than (S)-fluoxetine nevertheless has superior antidepressant effects and more consistently improves spatial learning and memory. Such a profile not only offers an advantage in treating depression but may also confer an additional beneficial effect in managing neurocognitive impairments associated with depression.

Disclosures: S. Marwari: None. **G.S. Dawe:** None.

Poster

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Support: NIH grant P50DA026306

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Title: Effects of HIV/TAT protein expression on spatial memory, reversal learning and neurotransmitter levels in mice

Authors: *S. G. SEMENOVA[1], J. P. KESBY[2], A. MARKOU[1];
[1]UCSD, La Jolla, CA; [2]The Univ. of Queensland, St. Lucia, Australia

Abstract: Background: Neurotoxic viral protein TAT may contribute to deficits in dopaminergic and cognitive function in individuals infected with human immunodeficiency virus. Transgenic mice with brain-specific doxycycline-induced TAT expression (TAT+, TAT- control) show impaired cognition. However, previously reported TAT-induced deficits in reversal learning may be compromised by initial learning deficits. We investigated the effects of

TAT expression on memory retention/recall and reversal learning, and neurotransmitter function. We also investigated if TAT-induced effects can be reversed by improving dopamine function with selegiline, a monoamine oxidase inhibitor. **Methods:** Mice were tested in the Barnes maze. TAT expression was induced after the task acquisition. Dopamine, serotonin and glutamate tissue levels in the prefrontal/orbitofrontal cortex, hippocampus and caudate putamen were measured using high performance liquid chromatography. Selegiline treatment continued throughout behavioral testing. **Results:** Neither TAT expression nor selegiline altered memory retention. On day 2 of reversal learning testing, TAT+ mice made fewer errors and used more efficient search strategies than TAT- mice. TAT expression decreased dopamine turnover in the caudate putamen, increased serotonin turnover in the hippocampus and tended to increase the conversion of glutamate to glutamine in all regions. Selegiline decreased dopamine and serotonin metabolism in all regions and increased glutamate levels in the caudate putamen. **Conclusions:** In the absence of impaired learning, TAT expression does not impair spatial memory retention/recall, and actually facilitates reversal learning. Selegiline-induced increases in dopamine metabolism did not affect cognitive function. These findings suggest that TAT-induced alterations in glutamate signaling, but not alterations in monoamines metabolism, may underlie improvements in reversal learning.

Disclosures: S.G. Semenova: None. **J.P. Kesby:** None. **A. Markou:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: CAPES

Title: Extrapolation of serial stimulus patterns is disrupted following selective damage to the anteroventral thalamus in rats

Authors: *D. G. SILVA, G. F. XAVIER;
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Abstract: Extrapolation of serial stimulus patterns, a type of anticipatory behavior, relies on memories of past regularities. It seems to depend upon the identification and application of rules relating sequences of stimuli, allowing anticipation of events never experienced before. According to Gray (1982) the septo-hippocampal system compares (1) anticipated information, predicted from memories of past regularities, motor programs in progress and prior experiences

on the relationship between execution of motor programs and environmental consequences, and (2) present information. The comparator would be the subiculum. This brain structure would receive present information from neocortical afferents, via the entorhinal cortex, and expected information from a "generator of predictions system" including the subiculum, mammillary bodies, antero-ventral thalamus, cingulate cortex and, again, the subiculum. The antero-ventral thalamus is in a privileged position to allow investigation of this postulated generator of predictions system. This study investigated the effect of selective damage to the antero-ventral thalamus, by topical application of N-Methyl-D-Aspartic acid (NMDA), on the ability of rats to extrapolate relying on serial stimulus patterns; Control subjects were injected with phosphate buffer. Wistar rats were trained to run through a straight alleyway to get rewarded. In each session (one per day) the rat run four successive trials, one immediately after the other, receiving different amounts of sunflower seeds in each trial. While rats exposed to the monotonic schedule received 14, 7, 3, 1 sunflower seeds along trials, subjects exposed to the non-monotonic schedule received 14, 3, 7, 1. Rats were trained along 31 sessions. On the 32nd testing session, a fifth trial never experienced before by all rats was included immediately after the fourth trial. Running times were recorded along trials. ANOVA including data of the testing session revealed a significant Trial x Surgery x Rewarding Schedule interaction effect ($F(4,96) = 6.83$; $P < .0001$). As expected, running times on the fifth trial of Control subjects exposed to the monotonic schedule were significantly longer as compared to the corresponding scores of Control subjects exposed to the non-monotonic schedule, indicating the occurrence of extrapolation. In contrast, lesioned rats exposed to the monotonic schedule did not exhibit this increase in running times on the fifth trial, indicating that these subjects did not extrapolate. In conclusion, results indicate that extrapolation relying on serial stimulus patterns is disrupted following selective damage to the antero-ventral thalamus.

Disclosures: D.G. Silva: None. **G.F. Xavier:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: Australian Research Council DE140101071

Title: High sucrose diets impair performance of a behavioral pattern separation mediated touchscreen task

Authors: *A. C. REICHELT[1,2], M. J. MORRIS[3], R. F. WESTBROOK[2];
[1]Sch. of Hlth. and Biomed. Sci., RMIT Univ., Melbourne, Australia; [2]Sch. of Psychology, [3]Sch. of Med. Sci., UNSW Australia, Sydney, Australia

Abstract: Rodent models have shown that high sucrose diets can reduce adult hippocampal neurogenesis. Functionally, neurogenesis is required for minimizing interference between memories that share features, a process that involves "pattern separation". We provided rats in the sucrose group with two hours daily access to a 10% sucrose (w/v) solution for 28 days and then assessed their performance on the "Trial-Unique Non-matching to Location" task (TUNL), conducted in a touchscreen chamber as a measure of behavioral pattern separation. In this task, rats first respond to a sample location and are then rewarded if they respond to a novel rather than the sample location in the choice phase. We varied the spatial separation between the novel and the sample location in order to manipulate the demands on pattern separation. Rats that had consumed sucrose performed the non-match discrimination correctly when there was a large spatial separation between sample and the new locations, but performed at chance levels when the separation was smaller. Doublecortin immunoreactivity in the dentate gyrus of sucrose-consuming rats was significantly reduced relative to controls. Thus, daily sucrose consumption impaired behavioral pattern separation performance and reduced measures of hippocampal neuroproliferation, confirming that diet can impair pattern separation and suggesting that this impairment is due to a reduction in hippocampal neurogenesis.

Disclosures: A.C. Reichelt: None. **M.J. Morris:** None. **R.F. Westbrook:** None.

Poster

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NIH NS087112

Title: Cognitive dysfunction in mice lacking of PKR-like endoplasmic reticulum kinase (PERK) in dopaminergic neurons

Authors: *F. LONGO, E. SANTINI, E. KLANN;
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Abstract: Accumulation of misfolded proteins can cause a pathological load on cells in the nervous system, resulting in endoplasmic reticulum (ER) stress that leads to the unfolded protein response (UPR) that typically is neuroprotective. A critical component of the UPR is PERK, whose activation results in the phosphorylation of eukaryotic initiation factor 2α (eIF2α), a translation factor that controls the initiation step of protein synthesis that has been implicated in long-term synaptic plasticity and memory formation (Ma et al., 2013). Although the UPR serves a neuroprotective role via regulation of general and gene-specific translation, it also is responsible for promoting apoptotic cell death following sustained activation, which occurs in neurodegenerative disorders such as Parkinson's disease (PD). Markers of PERK/eIF2α activation have been found in PD post-mortem brain tissue, where nigral dopaminergic neurons displaying α-synuclein inclusions also are positive for phosphorylated PERK and eIF2α (Hoozemans JJ et al., 2007). Dopaminergic signaling contributes to neuronal plasticity underlying learning and memory (Broussard JI et al., 2016), by acting upon specific neural targets such as hippocampus, amygdala, and prefrontal cortex, and altered dopaminergic modulation affects the encoding and maintenance of memories (Rosen ZB et al., 2015). Our central hypothesis is that a prolonged UPR results in inhibition of general protein synthesis via PERK-dependent phosphorylation of eIF2α, resulting in the loss of synaptic proteins, synaptic failure, and ultimately, neuronal death in dopaminergic neurons. To investigate the involvement of PERK in neurodegeneration and to determine whether the deletion of PERK in dopaminergic neurons results in synaptic alteration and cognitive symptoms, we generated mice selectively lacking either one or both copies of the gene that encodes PERK in dopaminergic neurons (DAT-PERK$^{+/-}$ and DAT-PERK$^{-/-}$respectively). We examined the DAT-PERK mutant mice in a series of cognitive tasks, including the Morris water maze, novel object recognition, Y maze, T maze, and associative fear conditioning and we found that selective PERK deletion in dopaminergic neurons results in multiple cognitive phenotypes in mice. In particular, DAT-PERK$^{-/-}$ mice exhibit behavioral impairments in both hippocampus- and amygdala-dependent memory resulting in a cognitive profile that recapitulates different features of PD. These results support our overall hypothesis and are consistent with previous studies that proper regulation of PERK-eIF2a signaling is required for normal cognitive function (Ma et al., 2013; Moreno et al., 2013).

Disclosures: F. Longo: None. **E. Santini:** None. **E. Klann:** None.

Poster

638. Learning and Memory: Rodent Studies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 638.15/III36

Topic: H.01. Animal Cognition and Behavior

Support: NIH-NIAP30

OCAST and PHF Seed Grant to F.D.

OCNS Translational Seed Grant to A.O

Title: Novel synaptic pathway in drug-resistant epileptic encephalopathy

Authors: *A. OROCK[1,2], S. LOGAN[1,2], Y. I. LEE[4], F. DEAK[2,3];
[1]Oklahoma Ctr. for Neurosci., [2]Reynolds Oklahoma Ctr. on Aging, Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK; [3]Geriatric Med., Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma city, OK; [4]Dept. of Anatomy, Col. of Med., Dankook Univ., Cheonan, Korea, Republic of

Abstract: Introduction: Ohtahara syndrome, also known as Early Infantile Epileptic Encephalopathy (EIEE) with suppression bursts is a currently untreatable disorder that presents with seizures and impaired cognition. EIEE patients have most frequently mutations in the gene encoding the synaptic protein of munc18-1. The exact molecular mechanism is unknown how these munc18-1 mutations cause seizures and cognitive impairment but the leading haploinsufficiency hypothesis posits that they make the protein unstable. This study aims to understand the role of munc18-1 and how targeting transmitter release could be used to combat EIEE and other drug resistant epileptic disorders.
Methods: We use munc18-1+/- mice as a model of EIEE haploinsufficiency. We performed behavioral tests for spatial learning and memory using a radial arm water maze (RAWM) and the "Intellicage" behavioral platform. We performed long-term potentiation (LTP) tests to assess hippocampal memory acquisition and storage capacity of munc18-1+/- mice. We used a fluorescent FM dye assay to measure the rate of synaptic vesicle release in neuronal cultures from munc18-1+/- and wild-type controls. We created viral vectors with point mutations found in EIEE patients to transduce cultured neurons and tested the effect of these mutations on neurotransmission.
Results: These behavioral assays show that munc18-1+/- mice perform poorly in spatial learning tasks (RAWM) and reversal learning task when compared to controls (Intellicage). They also have impaired LTP and slower synaptic vesicle release rates than controls. None of the selected Munc18-1 point mutations have a dominant negative phenotype but are able to partially rescue synaptic function in munc18-1+/- neurons.
Conclusions: To our best knowledge this is the first study to demonstrate that munc18-1 mutants implicated in the severe epileptic encephalopathy of EIEE are functionally less active than ^{WT}munc18 and hence could be a possible therapeutic target for drug resistant epileptic disorders.

Disclosures: A. Orock: None. **S. Logan:** None. **Y.I. Lee:** None. **F. Deak:** None.

Poster

638. Learning and Memory: Rodent Studies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 638.16/III37

Topic: H.01. Animal Cognition and Behavior

Support: China 973 program 2013cb835100

Title: Hippocampal activation of Rac1 regulates the forgetting of object-recognition memory

Authors: *Y. LIU, S. DU, B. LEI, L. LV, W. SHI, Y. TANG, L. WANG, Y. ZHONG;
Sch. of Life Sciences, Tsinghua Univ., Beijing City, China

Abstract: Passive or natural memory decay and interference-induced memory loss are two major forms of forgetting that are extensively studied in psychology. Whether and how these two forms of forgetting are regulated at the molecular level remain to be established for all types of memories in vertebrates. Recent studies in Drosophila led to the idea of active forgetting, as the behaviourally induced activation of the small G protein Rac1 causes forgetting. Here, we show that Rac1 activation in the mouse hippocampus plays a role in the regulation of time-based passive memory decay and interference-based forgetting of object recognition memory. Although it naturally decays within 72hrs, the inhibition of Rac1 activity in hippocampal neurons through targeted expression of a dominant negative Rac1 mutant extended memory for over 72hrs,whereas Rac1 activation accelerated memory decay within 24hrs. The recognition of additional objects led to interference-based forgetting of the original objects, and this interference-induced forgetting was blocked by the inhibition of Rac1 activity in hippocampal neurons. The evidence presented here suggests that Rac1 activation causes both time-based and interference-based forgetting of recognition memory, but exerts no effects on the formation of this memory. Indeed, the better-defined time window in the interference task allowed us to reveal interference-induced elevations in Rac1 activity. Electrophysiological recordings of long-term potentiation provided independent evidence that further supported a role for Rac1 activation in forgetting. Thus, Rac1-dependent forgetting is evolutionarily conserved from invertebrates to vertebrates.

Disclosures: **Y. Liu:** None. **S. Du:** None. **B. Lei:** None. **L. Lv:** None. **W. Shi:** None. **Y. Tang:** None. **L. Wang:** None. **Y. Zhong:** None.

Poster

638. Learning and Memory: Rodent Studies

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 638.17/III38

Topic: H.01. Animal Cognition and Behavior

Support: NSC 102-2410-H-431-005-MY3

Title: Re-examination of reward comparison hypothesis: Roles of ventral tegmental area and periaqueductal grey matter on morphine-induced aversively conditioned taste aversion and rewarding conditioned place preference

Authors: *C. WU[1], A. HUANG[2];
[1]Keelung Hosp., Keelung, Taiwan; [2]Fo Guang Univ., Yilan County, Taiwan

Abstract: A conditioned solution (CS) is paired with the unconditioned stimulus (US), which induces aversively illness or nausea effects, to produce the suppression of the CS consumption, in termed as conditioned taste aversion (CTA). Later, abused drugs have also been shown to produce the similar CTA effect. Some researchers suggest that the suppression of the CS is due to the reward comparison between CS and US. The reward value of the CS outweighs from that of the US, and thereby animals decrease the CS consumption, in called as the reward comparison hypothesis. The purpose of the present study addresses this interesting issue to dissociate from rewarding and aversive effects of abused drugs. The rewarding ventral tegmental area (VTA) and the aversive periaqueductal grey matter (PAG) were determined to assess this dissociation between abused rugs-induced CTA and CPP. Using “the pre- and post-association” experimental paradigm, we simultaneously form morphine-induce CTA in the pre-association and morphine-induced CPP in the post-association experiments. Before the CTA and CPP acquisition phase, the VTA (i.e., rewarding nucleus) and the PAG (i.e., aversive nucleus) were respectively lesioned. The 0.1% saccharin solution (CS) and the conditioned place preference were conditioned with morphine for 5 days paired and 5 days unpaired schedules. Finally, the CTA and CPP were tested for 15 min and 10 min, respectively. The results showed that VTA lesions decreased CPP effects but did not affect CTA. PAG lesions increased CTA effects but did not affect CPP. The present findings support the CTA was involved in the aversion and CPP was to control the reward. The VTA did not mediate aversive CTA effects and control rewarding CPP. The PAG did not mediate rewarding CPP effects and govern CTA effects. The reward comparison hypothesis is still challenged. Keywords: reward comparison hypothesis, conditioned taste aversion, rat NSC 102-2410-H-431-005-MY3

Disclosures: C. Wu: None. **A. Huang:** None.

Poster

638. Learning and Memory: Rodent Studies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 638.18/III39

Topic: H.01. Animal Cognition and Behavior

Title: Classic wide-waveform dopamine neurons signal reward number and identity error signals in a common framework

Authors: *Y. K. TAKAHASHI, G. SCHOENBAUM;
NIDA/NIH, Baltimore, MD

Abstract: Midbrain dopamine neurons have been shown to signal errors in reward prediction. This signal is thought to reflect the discrepancy between the value of expected and actual reward. We recently reported that error signaling of dopamine neurons in rat ventral tegmental area (VTA) was altered by disruption of input from orbitofrontal cortex that was proposed to signal information about task states. This suggests, contrary to current theoretical accounts, that dopamine error signals might contain information about predicted identity or state as well as value. Consistent with this hypothesis, here we show that dopamine neurons signal such value-less prediction errors. Neural activity was recorded from putative dopamine neurons in rat VTA. Recordings were made in a simple odor-guided choice task in which different odor cues indicated that a flavored milk solution was available in one of two nearby fluid wells. During recording, we manipulated either reward number (1 or 3 drops) or identity (chocolate- or vanilla-flavored milk) to induce value-based or value-less errors in reward prediction, respectively. Consistent with previous work, dopamine neurons showed phasic changes in firing in response to changes in reward number. However the same dopamine neurons also increased firing when the identity of reward was changed unexpectedly. These increases in firing disappeared with learning, were observed despite the rats showing similar preferences for the two rewards, and were observed in the same neurons that showed suppression of firing on reward omission. These results suggest that midbrain dopamine neurons can signal errors in predicting features of reward other than its general value.

Disclosures: Y.K. Takahashi: None. **G. Schoenbaum:** None.

Poster

638. Learning and Memory: Rodent Studies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 638.19/III40

Topic: H.01. Animal Cognition and Behavior

Support: Office for Research and Innovation at the University of Oregon

Medical Research Foundation

Title: Reward expectation influences the activity of auditory striatal neurons during decision making

Authors: *L. GUO, W. I. WALKER, S. JARAMILLO;
Inst. of Neurosci., Univ. of Oregon, Eugene, OR

Abstract: Animals constantly adapt their behavior based on environmental conditions to optimize reward and avoid punishment. To understand how neural circuits implement flexible sound-driven choices, it is necessary to identify the brain regions involved in these behaviors, and how neural activity is influenced by the outcome of a choice. The rodent caudal striatum receives direct inputs from the auditory cortex and the auditory thalamus, as well as outcome-related signals from midbrain dopaminergic innervation. This region is therefore ideally situated to integrate sensory information with feedback about the outcome of an action (i.e., reward or punishment), and potentially plays a key role in action-selection. In this study, we investigated the effects of the expected magnitude of reward on neuronal activity in the auditory striatum. Water-restricted mice were trained in an auditory categorization task where they learned to associate different sound frequencies with different reward ports to obtain water. We changed the amount of water delivered on each reward port every 150-200 trials. Isolated single units from extracellular recordings displayed frequency-selective sound-evoked responses as well as movement-related activity. A comparison of neuronal activity from trials with different amounts of expected reward revealed that a subset of striatal neurons were modulated based on reward expectancy at different time periods during the task. Our findings pave the way for future investigation of the neural circuits underlying sound-driven choices based on reward.

Disclosures: L. Guo: None. **W.I. Walker:** None. **S. Jaramillo:** None.

Poster

638. Learning and Memory: Rodent Studies

Location: Halls B-H

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Program#/Poster#: 638.20/III41

Topic: H.01. Animal Cognition and Behavior

Support: NRF-2015R1A3A1063485

HI14C-1922-010014

Title: Behavioral differences among C57Bl/6 substrains

Authors: *Y.-S. LEE, M. KANG, H.-H. RYU;
Chung-Ang Univ., Seoul-City, Korea, Republic of

Abstract: Different strains of mice show distinct behavior and physiological phenotypes. However, the differences among substrains of C57BL/6 are not well appreciated. Here, we compared three substrains of C57BL/6 (C57BL/6JBomTac, C57BL/6NCrljOri, and C57BL/6NTacSam) in a series of behavioral tasks examining both general behavior, and learning and memory. In addition, we have also performed electrophysiological analyses including long-term potentiation (LTP) in the hippocampus, which is considered a cellular mechanism for learning and memory. We found that there are significant differences between C57BL/6JBomTac and the other two substrains in their behavioral phenotypes, including motor coordination, anxiety, and freezing in classical fear conditioning. However, the three substrains show comparable levels of hippocampal LTP. Our findings provide guidelines for choosing the most appropriate substrain for the intended studies.

Disclosures: Y. Lee: None. **M. Kang:** None. **H. Ryu:** None.

Poster

638. Learning and Memory: Rodent Studies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 638.21/III42

Topic: H.01. Animal Cognition and Behavior

Title: Hippocampal mapk activity in nf1 mice model shows abnormal circadian oscillation

Authors: *L. CHEN, T. SERDYUK, Y. ZHOU, W. LI;
Bio-X Institutes,Shanghai Jiao Tong Univ., Shanghai City, China

Abstract: Neurofibromatosis Type 1 (NF1) is a common neurological disorder caused by mutations in *NF1* gene, which encodes Neurofibromin, a p21Ras GTPase activating protein. Importantly, dysfunction of *NF1* causes learning disabilities and attention deficits. Previous study showed that the learning and memory deficits in mouse model of *NF1* ($nf1^{+/-}$) appear to be caused by excessive p21Ras/MAPK activity leading to impairments in mechanism of learning and memory. In addition, it is reported that the circadian oscillation of hippocampal MAPK activity may affect a number of physiological processes, as the persistence of long-term memories. In our study, the MAPK phosphorylation activity of the $nf1^{+/-}$ mice shows abnormal circadian oscillation compared with wild-type mice littermates. The level of hippocampal MAPK phosphorylation was significantly elevated during the night in *NF1* mice model. Besides, the recording of local field potentials from CA1 (at daytime and night) showed a difference in theta-frequency and alpha- frequency power spectral densities (PSD) in neurons between wild-type and $nf1^{+/-}$ mice. Our findings suggest that the MAPK activity rhythms may play an important role in memory mechanism; these data may provide new thoughts for elaborating the cognitive defects in *NF1* mice model.

Disclosures: **L. Chen:** None. **T. Serdyuk:** None. **Y. Zhou:** None. **W. Li:** None.

Poster

638. Learning and Memory: Rodent Studies

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Topic: H.01. Animal Cognition and Behavior

Support: NIH T32007262

Title: Effects of mPFC inactivation during learning of an initial strategy on subsequent behavioral flexibility

Authors: *G. J. PETERS, K. M. LATTAL;
Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: The rat medial prefrontal cortex is a critical node in the neural circuitry that mediates behavioral flexibility. Notably, damage to the mPFC does not impair the learning of relatively novel memory-guided strategies, but in contrast, impairs learning new behavioral strategies that

conflict with existing strategies. Thus, the emphasis in research on behavioral flexibility is typically on the functional role of mPFC during the switch to alternative strategies. However, evidence from fear conditioning indicates that subregions within the mPFC have dissociable roles when learning conflicting behaviors. Here, prelimbic cortex (PL) is important for learning and expressing an initial fear memory, and infralimbic cortex (IL) is needed for learning a competing extinction memory. Additionally, PL and IL appear to play unique roles in other forms of competitive memory processes, such as the implementation of goal-directed or habitual strategies, respectively. Given prevalent bidirectional connections between PL and IL, one intriguing possibility is that these regions contribute to general flexibility by somehow interacting to resolve the conflict between alternative behavioral strategies. If so, mPFC involvement during the acquisition of initial strategies, though often undetected in behavior, might include the encoding of memory features that guide behavioral options in the event that flexibility is required. Consistent with this idea, it has previously been observed that temporary inactivation of the mPFC only during the early stages of learning a set of discriminations paradoxically improved learning on a new competing set of discriminations, despite several sessions of equivalent performance between control and previously inactivated groups on the initial set. In the current study, we assessed whether a similar inactivation procedure would result in improved flexibility in other forms of strategy switching. We found that temporary inactivation of the mPFC at the beginning of acquisition of a visual-cue strategy again improved performance on the switch to a competing response-based strategy, despite no difference in performance during the final sessions of the initial strategy. The robust nature of this facilitatory effect indicates that processing in the mPFC during the initial stages of learning has an important influence on subsequent flexibility. Such results have implications for understanding the neural circuitry dynamics that coordinate flexible behavior, which may ultimately inform approaches to treating the numerous psychiatric disorders that involve impaired flexibility.

Disclosures: **G.J. Peters:** None. **K.M. Lattal:** None.

Poster

638. Learning and Memory: Rodent Studies

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Topic: H.01. Animal Cognition and Behavior

Support: National Science Foundation - Graduate Research Fellowship Program

Human Frontiers

Title: Using trace eyeblink conditioning to uncover mechanisms of Presenilin-linked Alzheimer's Disease

Authors: *C. BLEVINS, J. SIEGEL, R. GRAY, B. V. ZEMELMAN;
Inst. for Neurosci., Univ. of Texas at Austin, Austin, TX

Abstract: Trace conditioning is a highly conserved form of forebrain-dependent learning in vertebrates. Much of the circuitry required for this cognitive task has been described, but roles of key circuit elements in learning are unknown. Moreover, variation in task performance can signal brain dysfunction. We have discovered a difference between 3xTg Alzheimer's disease (AD) model mice and non-transgenic controls in acquisition of trace eyeblink conditioning, a finding that extends to the mutant Presenilin-1 (mutPS1, M146V) knock-in model. PS1 encodes a multifunction protein that participates in neuronal calcium regulation and amyloid precursor protein (APP) processing. PS1 has gained prominence in AD after DNA genome analyses revealed that nearly 200 distinct mutations in this gene are causally linked to the most severe early-onset form of the disease. Surprisingly, both types of young AD mice expressing mutPS1 (2-3 months old) conditioned more rapidly than non-transgenic or wild-type littermate controls. No learning differences were detected using the hippocampus-independent, 'delay' version of the task. Interestingly, the differential learning enhancement extended to contextual fear conditioning, as young mutant mice displayed enhanced freezing to context (hippocampus-dependent), but not to tone (hippocampus-independent). This novel finding represents a rare opportunity to examine cellular and circuit mechanisms linking PS1 to memory function. Two hypotheses may explain our findings: Accelerated learning could stem from intrinsic changes in affected neurons, a plasticity advantage at the outset that is potentially maladaptive over time; alternatively mutPS1 may preferentially affect specific cell types or brain regions, leading to network level compensatory changes that temporarily enhance trace learning. Both phenomena could be features of AD, expressed as long-term cognitive decline. Upcoming experiments will examine the relationship between mutant animal age and accelerated learning. In addition, to explore mechanisms by which mutPS1 affects task performance, we will test the role of altered cellular plasticity in learning and use selective mutPS1 expression to study regional and cell type-specific contributions to the uncovered phenotype.

Disclosures: **C. Blevins:** None. **J. Siegel:** None. **R. Gray:** None. **B.V. Zemelman:** None.

Poster

638. Learning and Memory: Rodent Studies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 638.24/III45

Topic: H.01. Animal Cognition and Behavior

Title: If you say freeze, I'll freeze with you...how learning from self-experience affects the response to the display of defence behaviors of others

Authors: *A. CRUZ, M. MOITA;
Champalimaud Ctr. For the Unknown, Lisboa, Portugal

Abstract: We have previously shown that rats freeze upon the display of freezing by con-specifics. In addition, this form of observational freezing requires prior experience with foot-shock, suggesting that freezing becomes an alarm cue through learning. We hypothesized that, during exposure to shock, rats associate their own defense responses with aversive stimuli. Consistently, we found that rats exposed to shock that did not experienced freezing do not respond to the display of freezing by their cage-mate. Exposure to shock may also contribute to observational freezing through stress-induced sensitization. To address this issue we compared corticosterone levels after different shock exposures that led or not to freezing, and found no differences in the strength of the stress response. Still, stress could facilitate observational freezing. Hence, we subjected rats to a forced swim session, which induced a very strong stress response. However, these animals, did not display observational freezing. One remaining question is whether different triggers of freezing as animals experience and aversive stimulus lead to the same response during the social interaction. To address this issue we are currently testing how exposure to a predator cues affects responses to freezing displayed by conspecifics.

Disclosures: A. Cruz: None. **M. Moita:** None.

Poster

638. Learning and Memory: Rodent Studies

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Topic: H.01. Animal Cognition and Behavior

Support: Princeton University funding to KM

HHMI Funding to CDB

Title: The role of orbitofrontal cortex in model-based planning in the rat

Authors: *K. J. MILLER[1], M. BOTVINICK[2], C. BRODY[3];
[1]Princeton Univ., Princeton, NJ; [2]Google DeepMind, London, United Kingdom; [3]Princeton University; Howard Hughes Med. Inst., Princeton, NJ

Abstract: Planning can be defined as a process of action selection that leverages an internal model of the environment. Such models provide information about the likely outcomes that will follow each selected action, and their use is a key function underlying complex adaptive behavior. Orbitofrontal cortex (OFC) is thought to be a key structure underlying this type of behavior, though its precise role remains unclear. In previous work, we have developed a two-step repeated-trials planning task for rats (adapted from Daw et al., 2011), and shown that orbitofrontal cortex is causally involved in planning behavior on this task, as well as presented preliminary results from single-unit recordings indicating encoding of planning-related variables (Miller et al., SFN 2015; Miller et al., in prep). Here, we examine the role of the OFC in model-based planning in more detail with further electrophysiology data as well as temporally precise optogenetic inactivations. We find that single units encode model-based value signals associated with outcomes, but not with choices, supporting the idea that OFC may be involved specifically in model-based learning (Schoenbaum et al., 2009), rather than model-based choice. Further supporting this idea, preliminary data from optogenetic inactivations suggest that the OFC plays a causal role in the task at the time of learning, but not at the time of choice.

Disclosures: **K.J. Miller:** None. **M. Botvinick:** None. **C. Brody:** None.

Poster

638. Learning and Memory: Rodent Studies

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Support: NARSAD Young Investigator Grant

Penn State Hershey Neuroscience Institute

Penn State Parkinson's Disease Research Fund

Title: Functionally selective D_1 agonists bias regulate spatial working memory in prefrontal cortex

Authors: *Y. YANG, X. HUANG, R. B. MAILMAN;
Penn State Univ., Hershey, PA

Abstract: Dopamine D_1 agonists have been shown to cause cognitive enhancement, but importance of different signaling (e.g., cAMP or β-arrestin) is not known. The current study examined two D_1 agonists, EFF0311 and CY208243, that differ somewhat in their D1 signaling bias. Specifically, EFF0311 was found to have much higher intrinsic activity in β-arrestin

recruitment than CY208243, whereas activation of cAMP synthesis was similar. These properties allowed estimation of whether functional selectivity for these two pathways influences D_1 effects on cognition. Neuronal activity was recorded in prefrontal cortex as rats performed a delayed alternation spatial working memory task in a T-maze. Neurons with sensitivity to outcome-related aspects of the task decreased their firing rate after administration of both EFF0311 and CY208243, but in dissimilar ways. Before achieved outcome (prospective encoding), EFF0311 was superior to CY208243 in enhancing similarity between correct and error outcome based on firing rates. Oppositely, after outcome (retrospective encoding), EFF0311 was superior to CY208243 in distinguishing error from correct based on firing rates. Also, compared to CY208243, EFF0311 increased local field potential oscillation mostly in the beta-band, implying enhancement of the neuronal network. Finally the behavioral performance was improved by both EFF0311 and CY208243, but EFF0311 quickened decision making although the enhancement of performance accuracy was similar. These data on regulation of spatial working memory in prefrontal cortex suggest that D_1 mediated β-arrestin recruitment may be important for the use of D_1 ligands in the treatment of memory-related disorders, although off-target actions requires further investigation.

Disclosures: **Y. Yang:** None. **X. Huang:** None. **R.B. Mailman:** Other; Richard Mailman has interests in issued and pending patents related to dopamine D1 receptor mechanisms that constitute a conflict of interest for which there is University oversight..

Poster

638. Learning and Memory: Rodent Studies

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NASA NNX07AP84G

NASA NNX12AB55G

NIH DA 016765

NIH DA 023555

NIH DA 007290

MH107945-02

Title: What are the cellular and molecular underpinnings of improved mouse hippocampal-dependent behavior after exposure to a Mars mission-relevant dose of space radiation? Another small step for mousekind...

Authors: *S. YUN, C. W. WHOOLERY, M. J. LUCERO, A. W. WALKER, D. R. RICHARDSON, R. P. REYNOLDS, G. PALCHIK, S. E. LATCHNEY, B. P. C. CHEN, S. G. BIRNBAUM, A. J. EISCH;
UT Southwestern Med. Ctr., Dallas, TX

Abstract: Space radiation consists of high atomic weight and energy (HZE) particles. Chronic low doses of HZE particles - as will be encountered on a deep space mission to Mars - may be detrimental to astronauts by diminishing hippocampal function and thus compromising mission success. Studies using ground-based space radiation with relatively young adult mice (2 months of age) show that HZE particles decrease hippocampal neurogenesis and performance on hippocampal-dependent tasks. However, it is unknown how HZE radiation influences the brain and behavior of "astronaut age" equivalent mice (6 months of age), or how it alters important hippocampal functions such as contextual discrimination and cellular and molecular signatures underlying this behavior outcome. To fill these knowledge gaps, C57BL/6J mice (6-month-old [Mature] mice) were exposed to HZE particles (56Fe, 600 MeV/n, 174.1 KeV/μm LET of a single exposure of 20 cGy [Non-fractionated], protracted dose of 20 cGy [Fractionated, 6.7 cGy x 3 days, 48h intervals], or 0 cGy [Sham]). Surprisingly, while Mature Fractionated and Non-Fractionated mice displayed normal locomotor activity and normal CFC, they also showed a dose-dependent increase in contextual discrimination ability (CDFC) compared to Sham. To elucidate the cellular or molecular signature contributing to irradiation (IRR)-induced improvement of hippocampal- dependent behavior in Mature mice, we first quantified the number of newborn neurons and DG interneurons, both of which regulate DG activity. However, neither was not correlated with improved pattern separation. Second, we performed unbiased screening using whole translationome sequencing with a BAC-TRAP (Bacterial Artificial Chromosome-Translating Ribosomal Affinity Purification) mouse line targeted to DG glutamatergic neurons. Relative to the translationome from Sham mice, DG glutamatergic neurons from IRR mice had increased activity-dependent global translation. Thus, HZE particle radiation unexpectedly improves pattern separation in astronaut-age equivalent mice and is accompanied with enhanced global translation in DG glutamatergic mice. We are currently performing functional tests with candidate genes to assess whether they are causal factors underlying the age-related improvement in contextual discrimination after HZE radiation exposure.

Disclosures: S. Yun: None. **C.W. Whoolery:** None. **M.J. Lucero:** None. **A.W. Walker:** None. **D.R. Richardson:** None. **R.P. Reynolds:** None. **G. Palchik:** None. **S.E. Latchney:** None. **B.P.C. Chen:** None. **S.G. Birnbaum:** None. **A.J. Eisch:** None.

Poster

639. Learning and Memory Encoding in Hippocampal Circuits

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

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Topic: H.01. Animal Cognition and Behavior

Support: HFSP RGY0088-2014

Title: Towards a principled decoding of hippocampal replay

Authors: *Y. TANAKA[1], A. A. CAREY[1], E. ACKERMANN[2], C. KEMERE[2], G. PEZZULO[3], M. A. VAN DER MEER[1];
[1]Psychological & Brain Sci., Dartmouth Col., Hanover, NH; [2]Electric and Computer Engin., Rice Univ., Houston, TX; [3]Inst. of Cognitive Sci. and Technologies, Rome, Italy

Abstract: Decoding analyses seek to determine how well a known variable, such as a sensory stimulus, can be estimated from neural data. This process can be optimized for minimum reconstruction error, which can be computed on the same data used to estimate the encoding model. Internally generated hippocampal sequences pose an interesting challenge for this framework: they are covert phenomena without a ground truth to measure decoding performance against, and the true encoding model is unknown. Decoding the content of these sequences is thought to provide access to the content of memory retrieval, consolidation and planning processes, but the output of such a process is sensitive to parameter choices. How can we choose these parameters in a principled manner in the absence of a ground truth? First, we optimized for generalization performance, i.e. decoding accuracy on data not included in the estimation of the encoding model. When run on place cell data from a T-maze task, leave-one-trial-out generalization performance was overall much worse than same-trial-tuning and average-tuning decoding performance. More importantly, the decoder parameters that resulted in optimal performance were different for the generalization-optimized decoder. These parameters, in turn, can result in different decoded output of putative replay sequences. Second, to the extent that we can approximate internally generated sequences using a generative model, we can provide a ground truth that can be used for systematically choosing a decoder. We develop such a model that simulates replays by sampling from sped-up experience. The input to the model is a temporally compressed behavioral trajectory, the model parameters are derived from assumptions of the commonly used bayesian decoder, and the output an ensemble spiking pattern. If we are using the correct decoder, output of such a generative model should match experimental data. However, systematic examination of model parameters including temporal compression, replayed path length and number of encoded environments, show that data generated from our model based on these assumptions do not match the statistics of experimentally observed replays. Taken together, these results provide specific guidelines for a

more principled decoding of hippocampal sequences that can be straightforwardly applied to current decoding methods. In addition, the generative model approach provides a framework for the explicit testing of hypotheses underlying the spiking statistics and content of hippocampal sequences that be incrementally improved by comparison with experimental data and incorporated into decoding methods.

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Poster

639. Learning and Memory Encoding in Hippocampal Circuits

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Title: Species and strain differences in hippocampal region CA2 vasopressin and oxytocin receptor distributions in mice, rats and prairie voles

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Abstract: One of our research groups (WSY) determined that the knockout of the vasopressin (Avp) 1b receptor (Avpr1b) in mice resulted in decreased social aggression and decreased social memory in both male and females (Wersinger et al., 2002, 2007, 2008). We subsequently became interested in the hippocampal CA2 region when we determined that the Avpr1b was highly expressed there (Young et al., 2006). As we only found Avpr1b there in the Sprague-Dawley rat and C57Bl/6j mice, we (WSY) assumed that this would be the case in all strains of rats and mice. However, other groups (e.g., OJB; Bayerl et al., 2016) detected expression outside of the CA2 region in different rat strains. This prompted us to explore the expression of the Avpr1b in more depth as well as Avp receptor 1a (Avpr1a) and oxytocin (Oxt) receptor (Oxtr) in some cases in the hippocampus. Here were report initial results indicating that 3 rat strains have different patterns of expression whereas, so far, mouse strains do not. Finally, now not surprisingly, prairie voles have different patterns of expression. We used Affymetrix's very

sensitive ViewRNA *in situ* hybridization histochemistry system to examine expression. Both C57Bl/6j and BALB/c male and female mice express Avpr1b exclusively in pyramidal cells of the CA2 and immediately adjacent CA3 areas (expression is also seen in the fasciola cinereum which might be developmentally related). Oxtr is expressed within Avpr1b pyramidal neurons as well as in hippocampal interneurons and more extensively outside the hippocampus. In the Sprague-Dawley male, virgin female and lactating female rats, Avpr1b is found in the CA2 pyramidal cells as well as the SCN. In Wistar lactating female rats, Avpr1b is not expressed in the CA2 area but is in the SCN. No cell groups were identified expressing Avpr1b in the amygdala of either strain. In the male prairie vole, Avpr1b is not concentrated in the CA2 region although scattered cells are observed through the hippocampus. Strikingly, Oxtr is expressed in the same restricted CA2 pattern as Avpr1b in mice as well as abundantly elsewhere in the brain. Avpr1b, interestingly, is observed in many areas outside the hippocampus, especially in the globus pallidus and substantia nigra pars reticulata. Avpr1a is expressed in scattered cells in hilus and all strata in the dorsal hippocampus and along CA2 stratum oriens - pyramidal cell layers in the ventral hippocampus. Avpr1a is also expressed abundantly throughout the vole brain. These findings reflect the complex changes that evolve in the rodent neurohypophysial hormone receptor distributions despite the relatively fixed patterns of expression of the nonapeptides themselves.

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Poster

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Title: Hippocampo-thalamo-cortical networks for episodic memory and spatial processing

Authors: *N. A. KAMBI, J. M. PHILLIPS, Y. B. SAALMANN;
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Abstract: Episodic memory, the ability to remember objects, events and their contexts experienced in one's personal past, depends on a set of brain regions including the hippocampus, retrosplenial cortex (RSC) and anterior thalamus (ATN), which are highly interconnected with

each other. However, the precise nature of information transmission between the ATN, RSC and hippocampus in supporting episodic memory and spatial processing is largely unknown. Our study aims to characterize how the ATN communicates with the hippocampus and RSC during episodic memory recall and spatial transformations. We hypothesize that the ATN supports episodic memory and spatial processing by regulating information transmission between the hippocampus and RSC. A possible mechanism may involve the ATN synchronizing groups of neurons in the hippocampus and RSC according to episodic and spatial demands. In order to test our hypothesis, we perform diffusion MRI-guided multi-site electrophysiological recordings from interconnected sites of the ATN-RSC-hippocampus network. For this we measured network connectivity by acquiring high-resolution diffusion-weighted images (1 mm isotropic) from the 3T GE MR750 scanner with a 16-channel receive-only head coil in 6 anesthetized monkeys along 60 diffusion directions (b=1000 s/mm2 and NEX=14). We used FSL for EPI distortion, eddy current and motion correction prior to Bayesian estimation of diffusion parameters and probabilistic diffusion tractography (PDT). Our PDT results correspond broadly to that observed in neuroanatomical tracer studies. We observed overlapping projection zones of the hippocampus (subiculum) and RSC in the ATN, roughly corresponding to the ventral subdivision of ATN. Further, the likely medial subdivision of ATN connected with the hippocampus and medial cortical regions such as the posterior cingulate, anterior cingulate and orbitofrontal cortex. However, PDT data had the advantage of delineating projection zones specific to individual macaques, which cannot be precisely ascribed based on tracer data in the literature. Our data suggests that the ATN is well suited to regulate information transmission between the hippocampus and RSC, as well as medial cortex more generally, due to the indirect hippocampo-thalamo-cortical pathway. The connectivity map derived for each macaque allows us to target MRI-compatible laminar probes to interconnected sites in the hippocampus and RSC as well as their overlapping projection zones in the ATN, to monitor neural dynamics of the ATN-RSC-hippocampus network.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Title: Storing and retrieving pattern sequences in the hippocampal circuit

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Abstract: We recently proposed that episodic memories are best represented by temporal sequences of neural activation patterns and that the hippocampal circuit is optimized to store these sequences. One central feature in this theory, called CRISP, is that hippocampal area CA3 intrinsically produces temporal sequential activities through its abundant recurrent connections. Here, we first study the possible mechanisms by which a relatively fixed recurrent network structure in CA3 can generate neural activity sequences intrinsically. We find that the robustness of sequence generation depends on the network architecture. For instance, randomly connected recurrent networks (RCRNs) are very sensitive to noise, whereas locally connected recurrent networks (LCRNs) generate moderately robust neural sequences. Networks with sparse connectivity, which are trained with sequences of random patterns, exhibit the most robust dynamics. Next we implement a cortico-hippocampal circuit consisting of the EC-CA3-CA1-EC loop. During memory encoding, intrinsic CA3 sequences are hetero-associated with EC sequences, which are driven by sensory inputs. Downstream, CA3 sequences are hetero-associated with sequence in CA1 and finally, the CA1 patterns are hetero-associated with the sensory inputs in EC, thus closing the loop. During memory retrieval, intrinsic CA3 sequences have to be reactivated based on partial, noisy cues, which is provided to EC. Finally, the retrieved sequences in CA3 reactivate the stored sequences in EC via the CA1 layer.
We find that memory performance is determined by the network's ability to perform pattern completion of individual patterns as well as robust retrieval of sequential information from CA3. These two functions have competing requirements, i.e., the conditions that lead to good performance in one function might have detrimental effects on the other. For instance, modeling CA3 as a LCRN, the stored sequence is retrieved robustly, but the temporal correlations between patterns in the sequence impair pattern completion when decoding the CA3 pattern. On the other hand, a RCRN facilitates decoding, but sequence retrieval fails if any noise is present. Combining the advantages of each of these two models, networks pre-trained on sequences of random patterns achieve a good overall memory performance because they can encode sequences in CA3 robustly and be decoded in the feedforward connections to CA1 and EC. To conclude, we find that the cortico-hippocampal circuit shows high memory performance in storing and retrieving sequences of pattern, as hypothesized by CRISP, and that the network architecture of CA3 is critical for memory performance.

Disclosures: **M. Bayati:** None. **S. Cheng:** None.

Poster

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Title: Areal and cellular architecture of conserved cortical circuits in reptiles

Authors: *R. K. NAUMANN, G. LAURENT;
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Abstract: To understand cortical function at a mechanistic level, we need sufficiently simple animal models that retain basic structural elements of more complex systems such as the mammalian neocortex. Our laboratory is engaged in making such animal models accessible for modern molecular, imaging and electrophysiological techniques. The diversity of reptiles and their ancestral relation to mammals and birds allows us to explore questions related to the function and evolution of vertebrate neural circuits [1,2]. To facilitate physiological studies of cortical activity in reptiles, here we aim to provide a comprehensive anatomical map of reptilian cortical areas and document our findings using web-accessible high-resolution histological sections. We study two distantly related reptile species (Pogona vitticeps and Trachemys scripta) to identify shared and derived features of reptilian cortical circuits. We use a panel of methods for circuit mapping: viral and small-molecule tracing, chemo-architecture, gene-expression, and total area cell counts. Using immunohistochemistry and in-situ hybridization for a number of regulatory (Prox1, Lmo3, Rorb, Nurr1, Satb2) and functional (Drd1, Ndst4, Pcp4, Wfs1, CB) principal cell markers we identify a large number of region specific expression patterns in reptiles. We relate these patterns to specific intra- and extra-cortical projection patterns. These comparisons can suggest function, e.g., by comparison with the mammalian hippocampal formation.
Some markers have been claimed to identify specific cortical or pallial regions; we often find them more widely expressed than previously reported. Many homologies between vertebrate cortical areas have been proposed, but few agree on specifics [3]. Most comparisons have been based on distantly related birds and mammals while reports on reptiles are relatively rare. Our data inform this discussion by identifying regulatory gene expression specific to cortical areas and relating it to functional protein expression and cortical connectivity. We conclude that precise one-to-one similarities between reptile, bird, and mammalian cortical regions may exist, but that they are an exception rather than the rule. However, by identifying common elements in the cortex of distantly related reptiles (or similar emergent properties [1]) we may learn about

general cortical circuit motifs that were expanded and modified in evolution.
[1] Shein-Idelson et al., 2016 [2] Naumann et al., 2015 [3] Striedter, 2016

Disclosures: **R.K. Naumann:** None. **G. Laurent:** None.

Poster

639. Learning and Memory Encoding in Hippocampal Circuits

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Howard Hughes Medical Institute

Title: Entorhinal cortical drive distinguishes CA1 pyramidal neuron subpopulations

Authors: ***A. V. MASURKAR**[1], K. V. SRINIVAS[2], D. H. BRANN[2], R. A. WARREN[2], D. C. LOWES[2], S. A. SIEGELBAUM[2];
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Abstract: Although hippocampal CA1 pyramidal neurons (PNs) have long been thought to comprise a uniform population, recent evidence supports the existence of two distinct sublayers along the radial axis, with deep layer neurons more likely to form place cells, encoding spatial locale, compared to superficial neurons. Here we explore the potential circuitry that mediates the differential information processing in deep compared to superficial cells, using in vitro electrophysiology, two-photon imaging of spines, and optogenetics. Our results demonstrate that the direct inputs to CA1 from medial entorhinal cortex (MEC), which provide spatial information, elicit larger excitatory synaptic responses in deep compared to superficial CA1 neurons. Surprisingly, inputs from lateral entorhinal cortex (LEC), which provide nonspatial information, excite superficial CA1 neurons more strongly than deep neurons. Our experiments thus reveal a novel view of EC-dorsal CA1 connectivity, in that MEC and LEC engage CA1 PNs subtypes in different manners, and provide an important framework to understand in vivo network dynamics in different behavioral contexts.

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Title: Fragile X model mice show deficits in an a novel episodic memory task

Authors: *B. M. COX, W. WANG, A. LE, L. AGHAZADAH, J. HOU, G. LYNCH, C. M. GALL;
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Abstract: Fragile X syndrome (FXS) is the most common inherited cause of autism and mental retardation. In FXS expansion of an FMR1 gene CGG trinucleotide repeat disrupts expression of fragile X mental retardation protein and prior work has shown that Fmr1 knockout (KO) mice, used to model this disorder, have disdurbances in learning in many paradigms. In studies of acute hippocampal slices we find that the Fmr1 KOs have severe deficits in long-term potentiation (LTP) in the lateral perforant path (LPP) projections which arise in the lateral entorhinal cortex (LEC) and innervate the outer molecular layer of the dentate gyrus (DG). The LEC is the most densely interconnected of cortical regions and provides one of the two major cortical inputs to hippocampus. Animal and humans studies indicate that the LEC is critical for the formation of episodic memory (the 'what', 'where', and 'when' of serial events). Thus, we tested the hypothesis that Fmr1 KOs have deficits in LPP-dependent episodic memory. We developed a behavioral task in which mice sampled pairs of different odors in 3 successive trials and then on a 4^{th} test trial they were presented with an odor from the 1^{st} trial (a familiar odor) paired with a novel odor: greater time spent exploring the novel than the familiar cue during the test trial was considered evidence of learning. To show that learning in this serial odor recognition task (SORT) requires the LPP we used the designer receptor specifically activated by designer drug (DREADD) technology to transiently inactivate the LEC during training. Specifically, the AAV construct (AAV-CaMKII-HA-hM4D(Gi)-IRES-mCitrine) was injected bilaterally into LEC of WT mice and testing began 3 weeks later. DREADD agonist clozapine-N-oxide CNO (5 mg/kg or vehicle) was injected (IP, 30 min prior to behavioral testing). Inactivation of the LPP comletely blocked SORT learning, indicating that encoding relied upon LEC function. Finally, Fmr1 KO mice exhibited markedly impaired SORT learning. These results indicate that *Fmr1*

KOs have clear impairments in an LPP-dependent episodic memory task. Future work will examine the integrity of 'where' and 'when' components of episodic memory in the Fmr1 KOs.

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Poster

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Title: Functional neuroanatomy of nucleus incertus networks: neurochemical diversity, neural targets and control of cognitive, affective and motivated behaviors

Authors: *A. L. GUNDLACH, E. K. E. ONG-PÅLSSON, C. E. SINGLETON, V. RYTOVA, A. SABETGHADAM, M. HAIDAR, C. M. SMITH, R. A. D. BATHGATE, S. MA;
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Abstract: The brainstem *nucleus incertus* (NI) contains a highly-conserved population of GABA/peptide projection neurons in rodent, primate (and human) brain, and studies in rats and mice suggest their putative roles in control of stress, arousal and emotion- and motivation-related behaviors [see Ma S, Gundlach AL. J Neuroendocrinol 27, 457-67, 2015]. Evidence for these functions includes observations that the rat NI receives strong CRF/CRF1 receptor signals [Ma S et al. J Physiol 591, 3981-4001, 2013], and an orexinergic innervation from lateral hypothalamus and strong OX2-mediated activation [Blasiak A et al. Neuropharmacology 99, 432-47, 2015; Sabetghadam A et al. this meeting]. Similarly, chemogenetic activation of rat NI networks with an excitatory DREADD produced sustained, heightened arousal, and increased locomotor activity and vigilance [Ma S et al. Brain Struct Funct, in press].Therefore, the aim of our ongoing studies is to further characterize the functional neuroanatomy of the NI and the nature of its

neural targets in key circuits such as the septohippocampal system, basal forebrain and prefrontal/cingulate cortex, and limbic hypothalamus and thalamus; in relation to the impact of alterations in NI and relaxin-3 neuron activity and signaling on specific behaviors. In viral-based anterograde neural tract-tracing studies, we identified strong projections ascending to major midbrain, tectal, hypothalamic, thalamic, septal and cortical regions, and descending outputs to vestibular, oculomotor and autonomic centers. Quantitative analysis of comparative immunohistochemical studies of the rat NI, confirmed that the majority of neurons in the NI are GABAergic, of which ~50% produce relaxin-3; and identified a separate group of cholecystokinin-positive GABAergic neurons that lack relaxin-3. In an important discovery, relaxin-3 NI neurons were demonstrated to exclusively express TrkA, the high-affinity receptor for nerve growth factor (NGF). We also assessed the relative proportions of NI neurons delineated by calcium-binding proteins, calbindin and calretinin. Further immunohistochemical studies identified close contacts between relaxin-3-positive fibres and populations of somatostatin- and NGF-positive neurons in hippocampus (CA1, CA3, dentate gyrus). These findings emphasize the functional diversity of NI neurons and identify a key relationship between relaxin-3 and NGF/TrkA signaling systems, with implications for our understanding of how these GABA/peptide neuronal networks regulate cognitive processing, in relation to optimal levels of arousal, attention and motivation, under normal and pathological conditions.

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Poster

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Title: Attenuated hippocampal CA1 pattern reactivation and replay in rats with medial entorhinal cortex lesions

Authors: *A. CHENANI[1], M. SABARIEGO[2], M. I. SCHLESIGER MACLAREN[2], J. K. LEUTGEB[2], S. LEUTGEB[2,3], C. LEIBOLD[1,4];
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Abstract: During awake immobility and slow wave sleep, CA1 cell populations are active during brief bursts. The spiking of multiple cells within these bursts resembles sequences that correspond to place field sequences during running on a track or in a maze. Some of the events that correspond to those in behavior are already detectable before the beginning of a behavioral session (preplay), but corresponding events are generally most frequent during the period after a behavioral session (replay). The highly organized spontaneous hippocampal activity patterns during immobility and sleep are generally thought to reflect intrinsic hippocampal synaptic connectivity, and whether they are shaped by experience during behavior on the track or reflect innate intra-hippocampal wiring is currently debated. We previously reported that MEC-lesioned rats exhibit temporally disrupted place field firing, i.e. largely a loss of phase precession and pairwise correlation. Since such timing cues are assumed to be a main driving force for synaptic plasticity in vivo, we assumed that experience-dependent alterations of the synaptic matrix would be impaired in these lesioned rats. To test this hypothesis, we recorded hippocampal neuronal activity during sleep before and after running on a track or a figure-8 maze in control rats and in rats with extensive bilateral lesions of the medial entorhinal cortex (MEC). As previously reported, control rats displayed numerous events that correspond to maze trajectories, in particular during the sleep session after behavior, as measured by correlation to a template sequence of place fields. In rats with MEC lesions, we found that sequence replay was still significantly present but was strongly reduced compared to control rats. Furthermore, average activation of neuronal assemblies was measured by weighted projections to the significant principal components of the population vectors. Among rats in the control group, average assembly activation was higher in sleep following rather than sleep preceding the linear track sessions; such an increase was not observed in any of the lesioned rats. Our findings show that sequence replay and assembly reactivation strongly but not exclusively depend on intact MEC inputs.

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Poster

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Title: Functional characterization of recurrent networks within superficial layers of the medial entorhinal cortex

Authors: *I. ZUTSHI[1], M. FU[1], G. DE GUIA[1], V. LILASCHAROEN[1], J. K. LEUTGEB[1], B. K. LIM[1], S. LEUTGEB[1,2];
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Abstract: Spatial memory and navigation are thought to depend on the location-selective firing of various spatially modulated cell types, including grid cells. Grid cells are most abundant in the superficial layers (layer II, III) of the medial entorhinal cortex (mEC). Because of this overrepresentation in layers II and III, most theoretical models assume that local microcircuits within the superficial layers contribute to the generation of grid patterns. We therefore first characterized the extent of anatomical connections within the superficial layers of the mEC. Using anterograde and retrograde viral tracing techniques, we demonstrated that there is substantial anatomical excitatory connectivity between layer II and III and within layer II of the mEC, in particular within modules along the dorso-ventral axis. Next, we determined the effects of manipulating layer II pyramidal cells onto other cells within the superficial cell layers. To selectively target the mEC layer II pyramidal cells, we used a transgenic mouse line expressing cre recombinant protein under the wolfram syndrome 1 (*wfs1*) promoter and expressed channelrhodopsin in these cells. By manipulating *wfs1*+ cells while performing *in vivo* single-unit recordings in freely moving mice, we confirmed that there is both excitatory and inhibitory functional connectivity within the superficial layers. We also confirmed that both wfs1+ and wfs1- cells heterogeneously include non-spatial cells, spatial cells and grid cells, consistent with previous reports that there is no direct correspondence between known anatomical and functional cell classes. Based on these results, we sought to address to what extent the local connectivity of *wfs1*+ cells contributes to the emergence of grid cell firing patterns. Optogenetic manipulation of

wfs1+ cells gave rise to instant synchronous activation followed by a sustained inhibition across the majority of cells within the superficial mEC layers. Surprisingly, this broad local inhibition within the mEC had no effect on the spatial firing patterns of grid cells. These results suggest that while recurrent circuits exist within superficial layers of the mEC, such connectivity might not be necessary to support spatial representations. Instead, afferent projections to the superficial layers might already contain critical information that is sufficient to give rise to grid cell firing.

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Poster

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Title: Reorganization of the medial entorhinal spatial map is not a prerequisite for hippocampal global remapping

Authors: M. I. SCHLESIGER MACLAREN[1,3], B. L. BOUBLIL[1], J. B. HALES[4], J. K. LEUTGEB[1], *S. LEUTGEB[1,2];
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Abstract: A large number of everyday experiences can be stored in memory without interference between these memories. The core circuitry for such a large-capacity episodic memory system includes the hippocampus and the medial entorhinal cortex (mEC). One of the key functions of this circuitry is the rapid generation of neuronal representations that are highly distinct from those for previously acquired memories, a process referred to as pattern separation. For contexts with minor differences, pattern separation depends on intra-hippocampal processing. Distinct contexts, in contrast, are distinguished by reorganized spatial maps in the mEC, which are thought to mediate the orthogonalization of spatial maps in hippocampus. This process is referred to as global remapping. Because changes in medial entorhinal firing patterns are concurrent with hippocampal global remapping, it was hypothesized that computations in the

mEC are a prerequisite for the formation of distinct hippocampal maps. In support of this theory, it was recently found that the partial, acute inactivation of the mEC induced remapping in the hippocampus (Miao et al., 2015; Rueckemann et al., 2016). However, while these data demonstrate that changing mEC input is sufficient to trigger hippocampal remapping, it remains unknown whether mEC is necessary to perform this function. To address this question, we performed bilateral, excitotoxic lesions of the mEC and recorded hippocampal neural activity as rats explored a familiar and a novel environment. We found that highly distinct spatial maps emerged rapidly after exposure to a novel environment, in both control rats (CON) and rats with extensive lesions of the superficial layers of mEC. Consistent with global remapping in both groups, the maps were randomly reorganized between rooms (median spatial correlation, CON: -0.04, mEC lesion: -0.09, chance: -0.03, all P-values ≥ 0.35), and similarity between rooms was much lower than baseline, which was established by repeated recording sessions within the same room (CON: 0.89, mEC lesion: 0.65, all P-values < 0.001). Importantly, global remapping across rooms was found to be intact in each mEC-lesioned rat, even in individuals with no detectable sparing of the superficial layers. Our findings suggest that the emergence of distinct hippocampal maps does not require inputs from specialized cell types in the mEC. Alternative pathways that may support the generation of distinct hippocampal maps include the lateral entorhinal cortex and/or the medial prefrontal cortex, both of which have been shown to distinguish between different contexts.

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Poster

639. Learning and Memory Encoding in Hippocampal Circuits

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

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Topic: H.01. Animal Cognition and Behavior

Support: NIH R21 MH100354

NIH R01 NS084324

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Title: Optogenetic stimulation of parvalbumin neurons in the medial septum paces theta frequency and disrupts spatial memory

Authors: *C. R. QUIRK, M. K. WRIGHT, D. F. PARSEY, G. L. DE GUIA, J. K. LEUTGEB, S. LEUTGEB;
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Abstract: The hippocampus and medial entorhinal cortex (MEC) exhibit prominent oscillations in the theta range with a frequency of approximately 6-10 Hz during movement. Theta oscillations have been hypothesized to coordinate the precise timing of cell assemblies in the hippocampus and MEC and have been suggested to support episodic memory. Inactivating the medial septal area (MSA) results in a substantial reduction of theta amplitude in the hippocampus and MEC and in substantial impairment of hippocampus-dependent memory. These findings indicate that MSA plays a key role in generating theta oscillations as well as the precise spike timing that supports memory processing. We therefore asked whether offsetting the timing by manipulating the oscillation frequency would result in memory deficits. To gain control over theta, we manipulated the medial septal parvalbumin-expressing GABAergic (PV) cells. During endogenous theta generation, PV cells discharge in rhythmic bursts that are phase locked to theta frequency. To gain control over the discharge frequency of these cells, we used a cre-dependent viral vector (AAV.EF1a.DIO.ChR2.eYFP) to selectively express channelrhodopsin (ChR2) in PV cells in MSA of PV-cre mice. We then performed LFP recordings in the MEC to confirm that rhythmic stimulation of PV cells in MSA directly controlled the frequency of theta oscillations in MEC. The stimulation reliably elicited oscillations within the endogenous theta frequency range (e.g., 8 and 10 Hz) as well as outside the normal range (e.g., 12 and 20 Hz). Moreover, this stimulation protocol did not only allow for temporal control of theta but was also immediately reversible. To address the functional role of theta oscillations in memory, mice were trained on a spatial alternation task in which animals alternated between left and right sides of a figure-8 maze. On a subset of trials the animals ran continuously between left and right arms whereas in additional trials a 2-s or 10-s delay was imposed on the central arm. Once animals reliably performed each task (>80 % correct), optogenetic stimulation occurred on half of trials on alternating blocks. Stimulating at 10, 12, and 20 Hz significantly impaired performance on 2-s and 10-s delay trials compared to no stimulation in the same subjects. Control green stimulation, which weakly activates ChR2 did not impair performance in the task, suggesting the impairment was not a result of perturbation of behavior by light. These results demonstrate a functional role for endogenously paced theta oscillations in memory and that minor disruptions in the timing of oscillations are sufficient to cause a memory impairment as severe as a hippocampal lesion.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: R21 MH100354

R01 NS084324

Title: Impairments of hippocampus-dependent memory in mice with targeted expression of APP to CA3 principal neurons

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Abstract: Alzheimer's disease is a neurodegenerative disorder characterized by progressive mnemonic deficits. In humans, synapse and neuron loss is pronounced in the hippocampal formation, a region known to have an essential role for spatial learning and navigation. Transgenic mice that over-express human amyloid precursor protein specifically in CA3 pyramidal cells (CA3-3xTg) were used a model to investigate how amyloid-beta induced dysfunction of synaptic plasticity at Schaffer-collateral synapses affects hippocampal circuits and how this relates to spatial memory deficits. To test for memory deficits, we performed a spatial alternation task on a figure-8 maze with either no delay or brief delays (i.e., 2s or 10s) at the beginning of the center alley. The delay period is known to make the task dependent on hippocampal function while the continuous version is not hippocampus dependent. In 15-18 months old CA3-3xTg animals, we observed a decrease in the number of correct trials in only the hippocampus-dependent versions of the task. In order to understand changes in network function that may underlie this behavioral phenotype, we recorded local field potentials before, during, and after the spatial alternation task in CA3-3xTg mice and control littermates. In LFP recordings from CA1, we assessed the frequency and structure of sharp-wave ripple events (SWRs) during rest and behavior. SWRs are high frequency oscillations that are generated by the CA3 autoassociative network and are believed to support memory consolidation. We hypothesized that changes in SWRs could serve as a potential mechanism underlying cognitive impairments observed in CA3-3xTg mice. In control mice, SWR frequency during quiet restfulness is low before and increased after behavioral testing. In contrast, CA3-3xTg mice already showed elevated SWR frequency preceding the behavioral task and there was no additional task-related increase after behavior. We have also investigated spatial and temporal

firing patterns of hippocampal CA1, CA3 and DG neurons in order to complete our understanding of the effect of amyloid-beta induced toxicity on hippocampal processing. Taken together, our data indicate that synaptic toxicity restricted to CA3 results in substantial hippocampal network dysfunction and in impaired hippocampus-dependent behavior.

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Poster

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NIH R01 NS086947

Title: Bridging discontinuous events without hippocampus or medial entorhinal cortex: a behavioral and a physiological evaluation

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Abstract: The medial entorhinal cortex (MEC) projects directly to the hippocampus and is thought to be the key source of spatial and temporal information to hippocampal place cells. However, it remains unclear to what extent the spatial and temporal firing patterns in MEC are necessary for memory-guided behavior. We hypothesize that a primary function of MEC is to generate the temporal organization of hippocampal firing which could bridge gaps between discontinuous events. To test this, rats with either a hippocampal lesion, an MEC lesion, a double-lesion (hippocampus and MEC) or a sham lesion were trained to perform a continuous spatial alternation task in which the animals alternated between left and right sides of a figure-8-maze to receive reward. After animals reached criterion, blocks of trials were introduced with 2-second, 10-second, and 60-second delays. All groups performed similarly during the trials without delay, but when a delay was imposed, the lesion groups made significantly more errors. After further training, the single lesion groups improved their performance with short delays (2

and 10-seconds) but showed a long-lasting impairment under the 60-second condition. Double-lesions prevented the partial recovery in the short delay conditions and promoted a different pattern of response, specifically during long delays, in which these animals exhibited a tendency to continue choosing one side of the maze even if the task required them to alternate. Recordings of hippocampal single units and local field potentials were performed during the spatial alternation task to investigate the mechanisms that could support the spared memory function in MEC-lesioned rats. Our results demonstrated that hippocampal spatial firing was detectable in MEC lesioned animals, but that the spatial firing was less consistent than in controls, similar to what we previously reported in tasks without memory demand. Taken together, these results suggest that even a highly degenerated hippocampal code can be used to partially support short-term memory and prevents maladaptive strategies such as perseveration on response options that no longer produce a desired outcome.

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Poster

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Title: Role of the medial entorhinal cortex in odor sequence learning in rats

Authors: ***J. B. HALES**, L. L. BENSTER, P. J. BRESLIN, A. CAMACHO, T. A. J. W. FISHER, A. E. MORSE;
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Abstract: The hippocampus is critically involved in processing both spatial and temporal aspects of memory. Although spatially selective cells, known as place cells, were first discovered in the hippocampus in the 1970s (O'Keefe and Dostrovsky, 1971), recent studies have also reported the existence of temporally selective cells in the hippocampus, known as time cells (MacDonald et al., 2011). In the adjacent cortical area of the medial entorhinal cortex (MEC), spatial memory processing and spatially selective neuronal firing have been reported (Steffenach et al., 2005; Hales et al., 2014). However, the involvement of the MEC in temporal aspects of memory processing are less understood. A recent study reported that MEC lesions substantially disrupt theta phase precession in the hippocampus, suggesting the involvement of the MEC in temporal

organization of hippocampal firing patterns (Schlesiger et al., 2015). In order to directly examine the involvement of the MEC in temporal memory processing, we adapted an odor sequence learning task in rats, which has previously been shown to be dependent on the hippocampus (Fortin et al., 2002). Rats with either complete bilateral excitotoxic lesions of the MEC or sham lesions are presented with sequences of 5 odors. Following a 3 minute delay, a sequential order test probes memory for which of the two odors was presented earlier in the sequence. An odor identity test probes item memory by comparing an odor from the sequence with an odor that was not in the sequence. Memory for sequential order, but not odor identity, has previously been shown to depend on an intact hippocampus (Fortin et al., 2002). Results on both memory tasks will be discussed in terms of MEC involvement in temporal aspects of memory processing.

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Poster

639. Learning and Memory Encoding in Hippocampal Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: NSERC

Title: Dopamine D2-type receptors in the dorsal hippocampus mediate social learning in female but not male mice

Authors: *R. MATTA, E. A. UNDERWOOD, Z. K. LEACH, A. C. VERTES, V. ATABAKHSH, M. B. DA SILVA, E. CHOLERIS;
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Abstract: Dopamine (DA) is involved in mediating motivationally relevant behaviors such as drug/alcohol addiction, food intake, and social behaviors. Previous work in our lab using systemic treatments has shown that D1-type (D1/D5) DA receptors are involved in social learning, whereas D2-type (D2/D3/D4) DA receptors are involved in feeding behavior in the social transmission of food preferences (STFP) in mice (Choleris *et al*, 2011). The ventral tegmental area (VTA) has DAergic projections to numerous limbic sites, including the septum, amygdala, nucleus accumbens, and hippocampus. In particular, the hippocampus has been previously established as an important structure in mediating the STFP. Work in our lab has shown that blocking D1-type DA receptors in the dorsal hippocampus impairs social learning in the STFP in both male and female mice (Matta and Choleris, 2014). In this study, we

investigated the role of D2-type DA receptors in the dorsal hippocampus in the STFP. We microinfused the D2-type DA receptor antagonist Raclopride (at 10, 14, 18 or 20 μg/μL) directly into the dorsal hippocampus of male and female CD-1 mice (young adults) 10min before a social interaction (where social learning occurs) with a same-sex conspecific. We found that Raclopride at 10, 18 and 20 μg/μL blocked social learning in female, but not male mice. Furthermore, the social learning impairment could not be explained by any secondary effects on feeding, since total food intake was unaffected by drug treatment. Moreover, an olfactory discrimination task using the two highest doses of Raclopride that also blocked social learning (at 18 and 20 μg/μL) showed that the social learning impairment could not be directly explained by any changes in olfaction. Thus, the female effects of dorsal hippocampal D2-type DA receptor blockade may have been specific to social learning. Our study is also attending to effects of drug treatment on various social and nonsocial behaviors during the social interactions, and possible effects of gonadal hormones. Thus, DA in the hippocampus promotes social learning differently in males and females: in males it acts through only D1-type DA receptors, whereas in females it acts through both D1-type and D2-type DA receptors.

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Poster

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HHMI

Title: Interactions between the dorsal and ventral hippocampus and the nucleus accumbens during adaptive spatial reward learning

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Abstract: The brain's ability to associate experiences with subsequent rewards is fundamental to learning and memory, yet the neural substrates of this process are only partially understood.

Substantial evidence points to the hippocampus (HPC) and nucleus accumbens (NAc) as constituents of a critical neural circuit for spatial reward learning. A candidate mechanism for strengthening spatial-reward associations is the hippocampal sharp-wave ripple (SWR), a network event that reactivates sequences of hippocampal place cells in a manner that often recapitulates prior experience. Previous work has shown that in sleep following behavior, some NAc cells are reactivated coincidentally with hippocampal SWRs, but it remains unclear how the representations of these NAc cells relate to the learned experience. Furthermore, prior studies focused on the dorsal hippocampus (dHPC), while the ventral hippocampus (vHPC) exhibits the most direct anatomical projection to the NAc and may be critical for adaptive behavior. We therefore sought to understand how interactions between the dHPC, vHPC, and NAc support flexible, spatially guided reward learning. We recorded simultaneous single unit activity and local field potentials from these three regions in awake, behaving rats learning a spatial working memory task with changing reward contingencies. We found that NAc neurons represented various task elements including reward, were often spatially modulated, and could adapt their firing patterns with the changing reward contingencies. Moreover, NAc neurons with distinct firing patterns were differentially engaged by dHPC and vHPC SWRs during both awake immobility and sleep. Our findings indicate that the HPC engages a heterogenous population of NAc neurons at the precise time of SWRs, and that the SWR-related activity in the NAc may reflect distinct types of spatial and task associations reflective of dHPC and vHPC input.

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Poster

639. Learning and Memory Encoding in Hippocampal Circuits

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Title: Reactivation of distinct representations of moving and still experiences across the hippocampal-cortical network

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Berkeley, CA; [4]HHMI, Kavli Inst. for Fundamental Neurosci. and Univ. of California, San Francisco, San Francisco, CA

Abstract: The hippocampus and the prefrontal cortex (PFC) are critical for transforming representations of experience into memories but the specific nature of the experiences that form memories remains unclear. The hippocampus maintains spatial representations of experience. During locomotion, hippocampal place cells with discrete place fields are activated sequentially as an animal moves through space. Previous work has shown that these sequential patterns reappear during hippocampal sharp wave-ripples (SWRs), which are hypothesized to support storage and recall of memory. Recently, our laboratory demonstrated that a distinct set of place cells signal spatial location during immobility. Whether these representations of experiences that occur during immobility are stored and reactivated during SWRs and how they manifest in the PFC is unknown. We simultaneously recorded from multiple single neurons in the prefrontal cortex and the hippocampus of awake, behaving rats to examine the interactions between these structures. We first found that immobility place cells do participate in SWRs, but strikingly, these SWRs tend not to engage place cells that are active during locomotion. Moreover, these two distinguishable patterns of reactivation are also seen in the PFC. Individual PFC cells show changes in firing rate during SWRs, but they are modulated differently depending on whether the hippocampal SWR reactivates representations associated with locomotion or immobility. These findings suggest experiences associated with states of locomotion and immobility are encoded as discrete and distinct types of memories in the hippocampal-cortical network.

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Poster

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Ken Kennedy Institute for Information Technology

Title: Scoring sequences of hippocampal activity using hidden Markov models

Authors: *E. ACKERMANN[1], K. MABOUDI[2], K. DIBA[2], G. PEZZULO[3], M. VAN DER MEER[4], C. KEMERE[1];
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Abstract: Much is still unknown about the mechanisms underlying learning and memory, as well as the functional significance of associated hippocampal replay sequences. There is strong evidence supporting the roles of these replay sequences in a number of cognitive functions, including memory consolidation and behavioral planning. However, a statistical understanding of replay events is still lacking.
Here, we extend our previous work on using hidden Markov models (HMMs) for the sequential analysis of neural data in a number of ways. First, we introduce a novel two-component sequence score which decouples the sequential and contextual information, leading to an elegant and effective way to discriminate sequentially consistent patterns of neural activity from putative 'noise'. Moreover, we show how we can use this score to identify the underlying context and behavioral state of an animal more effectively than using the log probability for a sequence in the model directly.
Second, we show that the model leads to behaviorally relevant and interpretable results for a wide range of model parameters, so that the problem of model selection is mitigated significantly. This is especially important in the context of hippocampal replay, where behavioral correlates are not available; in the absence of behavioral correlates, we necessarily have to rely more heavily on the model to reveal underlying patterns of sequential information.Third, we discuss a number of possible approaches to order and interpret the virtual place fields learned by the model, including orderings and state associations suitable for both linear and nonlinear (or generalized) environments.
Finally, we present some preliminary results when using HMMs for online sequential analysis of long continuous recordings of neural activity. The analysis of such long duration recordings is particularly important for a more comprehensive understanding of replay content. Indeed, replay is expected to occur throughout the day, presumably representing recent, past, distant past, and never-before-experienced experiences (such as shortcuts to reward locations), yet existing analyses of replay have mainly considered recording sessions shortly before and after short term (approximately one hour) behavioral experiments. As a consequence, much of what has previously been labeled as 'noise' might in fact be consistent with past experience, and analyzing long term chronic recordings of the animal's past promises to help us understand the content and statistical characteristics of replay events.

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Poster

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Title: A cortical-hippocampal-cortical loop of information processing during memory consolidation

Authors: *G. ROTHSCHILD[1], E. EBAN[2], L. M. FRANK[1,3];
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[2]Google Inc., Mountain View, CA; [3]Howard Hughes Med. Inst., San Francisco, CA

Abstract: The hippocampus is critical for forming memories of daily life events, but over time these memories can become independent of the hippocampus[1-3]. The dominant model for this memory consolidation process posits that following rapid memory encoding in the hippocampus, repeated reactivation of hippocampal representations during sleep transfers information from the hippocampus to the cortex for long term storage[4-7]. This unidirectional information transfer is thought to be driven by sharp wave ripple (SWR) events[7-14], which mark periods of coordinated reactivation in hippocampus and cortex[15-19] and have been shown to directly support learning[20-22]. SWRs are generated in the hippocampus[23-25], drive excitatory responses in cortical target regions[25], and modulation of cortical neural activity has been reported to follow SWRs[15-18,26], consistent with unidirectional hippocampus to cortical information transfer. However, some observations are hard to reconcile with this model, including demonstrations that modulation of cortical activity can also precede SWRs[27,28], and that sensory stimulation can bias SWR content[29]. Thus, the direction of information flow between the hippocampus and cortex underlying memory consolidation remains unclear. Here we show that during sleep, the cortex leads a rapid cortical-hippocampal-cortical loop of information flow. We simultaneously recorded neural activity in the auditory cortex (AC) and the hippocampal CA1 region of rats as they learned a sound-guided task and during interleaving rest sessions. We examined the structure of cortical and hippocampal activity during sleep and found that reactivation in AC precedes and predicts the subsequent content of hippocampal activity during SWRs, while hippocampal patterns during SWRs predict subsequent cortical activity. Furthermore, delivering

sounds during sleep biased AC activity patterns, and sound-biased AC reactivation predicted subsequent CA1 activity, providing a potential mechanism for sensory-stimulation-biasing of memory during sleep[29-33]. These findings indicate that the reactivation of specific cortical representations during sleep could be a critical determinant of which memories are consolidated into long term stores.

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Poster

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Title: Characterization of hippocampal replay across learning

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Abstract: Hippocampal replay events are thought to play an essential role in learning and memory. Individual replays can contain spiking patterns that reflect different categories of spatial trajectories, including sequences that are replayed in either a temporally forward or reverse order and sequences that can represent either the current environment or a previously experienced and spatially distinct environment. We also know that novelty, spatial learning and reward can modulate the prevalence of replay, but how these factors influence the direction and content of replay remains unclear.
We therefore developed a new statistical paradigm to categorize the content of hippocampal replays using a decision state point process filter. This filter simultaneously estimates the spatial

representation being replayed as well as the direction of movement and the spatial context. Here we apply this paradigm to classify the replay events of rats learning two novel spatial alternation tasks. We explore multiple different binary decision states, including the direction of movement and the local or remote nature of the representation. Our analyses allow us to characterize the frequency of specific types of replay events across specific phases of learning.

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Poster

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Title: Low latency, multichannel sharp-wave ripple detection in a low cost, open source platform

Authors: ***S. DUTTA**[1], C.-T. WU[2], D. LIU[4], M. KARLSSON[8], L. FRANK[5,6,7], M. VAN DER MEER[9], D. JI[2,3], C. KEMERE[1,2];
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Abstract: Sharp-wave ripples (SWR) are brief bursts of ~200 Hz oscillations in the local field potential in area CA1 of the hippocampus that co-occur with dense bouts of ensemble spiking. SWR and the concomitant neural activity are associated with aspects of memory consolidation, recall, and memory-guided decision making. Temporally-specific silencing of hippocampal activity triggered by online detection of SWRs has been shown to have significant effects on memory consolidation and working memory. However, the real-time signal processing systems used to perform these experiments have been challenging to implement and inaccessible to many

researchers. Here, we present a ripple-detection module written for an open-source software platform (Trodes - https://bitbucket.org/mkarlsso/trodes). In conjunction with low-cost open-source hardware (OpenEphys - http://www.open-ephys.org, < $5K) or a pre-packaged commercial solution (SpikeGadgets - http://www.spikegadgets.com, < $15K). We achieve low latency SWR detection across multiple electrodes during ongoing neural recording experiments. Our analysis of SWR detection for real and synthetic data makes clear that the latency and accuracy of any SWR detection system are jointly limited at a fundamental level, with minimal latency of ~40-60 ms. Using a synthetic "gold-standard" data set, we demonstrate that our online open-source system can achieve accurate detection with limited additional latency (~10-15 ms), with even lower values achievable using the same module with our commercial hardware. Finally, we describe how our module can be extended to further include other information such as behavioral traces or multiunit activity in the SWR detection process. At < $5K for 32 channels of neural activity, this is a cost effective and simple approach for researchers seeking to add this experimental technique to their studies. In general, we anticipate that the use of modular signal processing blocks will significantly facilitate the dissemination of causal/closed-loop experimental techniques that represent the frontier of systems neuroscience experiments.

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Poster

639. Learning and Memory Encoding in Hippocampal Circuits

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

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Topic: H.01. Animal Cognition and Behavior

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CAREER

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BRAIN EAGER

Title: Replay detection: a comparison between hidden Markov models and existing approaches

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Abstract: Formation of memory of a behavioral experience is facilitated through reactivation of neural ensembles representing that experience. Reactivation of place cells in the hippocampus following a behavioral experience have been widely studied. As the animal runs across a field, the associated place cells become active in a sequential manner. It has been shown that these sequences frequently re-occur in a time-compressed manner (so-called replay) during rest and sleep. Detection of these replay events and the extent to which their expression changes during sleep can reveal the dynamics of memory processing. Therefore, a reliable method of replay detection is of special importance. Different methods have been recruited so far to detect replay events, including rank-order correlation, template matching, and Bayesian decoding. Recently, a hidden Markov model (HMM) based approach has been proposed as another alternative. Using this model, it is assumed that temporal patterns of population activity reflect a number of underlying states and transitions between them. In the case of replay, these states most likely correspond to virtual places. Using HMMs for replay detection is promising because existing methods need explicit external position information along with the neural activity during behavior to detect replay events. However, HMMs allow for the detection of replay even when no explicit position information is available. In a preliminary analysis, we trained an HMM on behavioral data and evaluated its performance in replay detection. We found that the performance of the HMM for the detection of visually verified replay events is comparable to existing methods, and moreover, in some cases of noisy replay events, the new method outperformed others. Here, we will do a comprehensive analysis of the benefits and limitations of the HMM based approach in comparison with existing approaches.

Disclosures: K. Maboudi Ashman Kamachali: None. **E. Ackermann:** None. **H. Miyawaki:** None. **C. Kemere:** None. **K. Diba:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

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ERC AdG 250345

Title: Cellular mechanisms of sparse coding in hippocampal granule cells

Authors: M. P. CHRISTENSON[1], *C. SCHMIDT-HIEBER[2], H. WEI[3], M. HAUSSER[1]; [1]Wolfson Inst. for Biomed. Res., [2]Univ. Col. London, London, United Kingdom; [3]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: The hippocampus must form distinct neuronal representations of memory items in order to differentiate efficiently between similar events. Granule cells of the dentate gyrus, the first stage of the hippocampal circuit, have been proposed to perform this task in a process termed pattern separation. It is unclear how synaptic input patterns are decorrelated and converted into sparse action potential output during this process. To address this question, we performed whole-cell patch-clamp recordings from mature hippocampal neurons in head-restrained mice navigating in a virtual-reality environment. Granule cells depolarized by ~3 mV during running periods, with depolarization preceding running by up to 1.5 s. Membrane potential and running speed were tightly correlated, suggesting that granule cells may receive speed-modulated synaptic inputs. Voltage-clamp recordings revealed that mean excitatory current input increased during running, while the frequency of large multicomponent EPSCs decreased. To try to trigger plasticity, we used theta-burst current injections (5 100-ms current injections at 5 Hz). Following the induction protocol, we observed a persistent hyperpolarization of membrane potential by ~3 mV lasting up to 90 s. The extent of hyperpolarization was positively correlated with the number of evoked action potentials during theta-burst current injections. While most granule cells were silent throughout the recording duration, spatial modulation of firing could be induced by near-threshold sustained current injections, similar to previous findings in CA1 pyramidal cells (Lee et al., 2012). Unlike in CA1 pyramidal cells, spatial modulation was highly sensitive to depolarization, with the number and location of firing fields depending on the sustained current injection amplitude. Our results indicate that granule cells receive desynchronized, yet spatially specific inputs during navigation, and their output is sensitive to small changes in excitatory drive. Moreover, granule cells are suppressed in an activity-dependent manner, which may help sparsify the population activity and orthogonalize output patterns.

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Poster

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Topic: H.01. Animal Cognition and Behavior

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NSF IIS-1302256

Title: Biologically realistic spiking model of hippocampal circuitry for enhanced machine learning

Authors: *D. J. HAMILTON, D. W. WHEELER, S. VENKADESH, K. MORADI, C. L. REES, G. A. ASCOLI;
Krasnow Inst. for Advanced Study, George Mason Univ., Fairfax, VA

Abstract: The hippocampus is a highly conserved architectural component of the mammalian brain that has evolved to perform associative learning tasks. It stands to reason that the field of machine learning, which historically has utilized multi-layered artificial neural networks and, more recently, "Deep Learning" to characterize large datasets, will benefit from exploiting specific organizational and functional principles garnered from hippocampal circuitry. Using knowledge available in Hippocampome.org, it is now possible to construct spiking neural network (SNN) models of the rodent hippocampus. This highly curated open-access resource defines hippocampal neuron types primarily based on axonal-dendritic patterning and is informed through dense coverage of peer-reviewed literature, which documents experimental evidence. Upon the foundation of these neuron types, we layer information about connectivity, intrinsic electrophysiology, input/output firing pattern relations, and molecular markers that allow us to derive a biologically realistic SNN model of the hippocampal formation. We then attempt to leverage known hippocampal circuit functionalities, such as pattern separation and auto-association, to improve the generalization ability of SNNs for machine learning. The specific application selected to vet this approach and to frame-out the computational infrastructure is automated literature mining. Since we have ready access to thousands of manually reviewed articles from local lab-based neuroinformatic literature mining efforts, we can harness their associated "ground truth" for training and testing. Through improved automated analytics leveraging biologically realistic circuitry, we strive to enhance machine learning for the advancement of science.

Disclosures: **D.J. Hamilton:** None. **D.W. Wheeler:** None. **S. Venkadesh:** None. **K. Moradi:** None. **C.L. Rees:** None. **G.A. Ascoli:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

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Title: Large-scale genetic profiles of hippocampal principal neurons through Allen Brain Atlas mining

Authors: D. J. HAMILTON, C. M. WHITE, C. L. REES, D. W. WHEELER, *G. A. ASCOLI; George Mason Univ., Fairfax, VA

Abstract: Neurons are typically classified based on their morphological, molecular, and electrophysiological properties, but rarely by combining all three of these dimensions. The open-access online knowledge base Hippocampome.org primarily defines neuron types from the rodent hippocampal formation based on their main neurotransmitter (glutamate or GABA) and the locations of their axons and dendrites across canonical sub-regions and layers (e.g. CA1 statum oriens or entorhinal layer 2). Any and all published information regarding biomarker expression (and several electrophysiological features) that can be linked to identified neuron types is then added along with the supporting experimental evidence. The resulting biochemical profile is relatively sparse: even for the best studied neuron types the expression or absence of fewer than 50 molecules has been firmly established to date. The massive mouse brain gene expression analysis conducted by the Allen Institute provides a wealth of data that when appropriately interpreted can be leveraged to substantially augment the biomarker knowledge in Hippocampome.org. In this study, we restrict the investigation to the principal cell layers of dentate gyrus (DG), CA3, CA2, and CA1, where the vast majority of the neurons are glutamatergic projecting neurons. Thus, Allen Brain Atlas (ABA) in situ hybridization data for those layers can justifiably be linked to the respective principal neuron types (i.e. Granule in DG and Pyramidal in CA3, CA2, and CA1). We filtered the whole-genome expression dataset to maximize consistency with current Hippocampome.org biomarker content. The resulting additional set of expressed/not-expressed genes expands the ~1,100 Hippocampome.org biomarker pieces of knowledge (PoK) by more than an order of magnitude, yielding a considerably more complete genetic characterization of principal neuron types in the mouse hippocampus.

Disclosures: D.J. Hamilton: None. **C.M. White:** None. **C.L. Rees:** None. **D.W. Wheeler:** None. **G.A. Ascoli:** None.

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Title: Computationally efficient models capturing diverse hippocampal neuronal behaviors

Authors: *S. VENKADESH[1], A. O. KOMENDANTOV[1], D. J. HAMILTON[1], D. W. WHEELER[1], S. A. LISTOPAD[2], J. KRICHMAR[3], G. A. ASCOLI[1];
[1]Krasnow Inst. for Advanced Study, George Mason Univ., Fairfax, VA; [2]Henry Samueli Sch. of Engin., [3]Dept. of Cognitive Sci., Univ. of California, Irvine, CA

Abstract: Simulation costs of biophysically detailed Hodgkin-Huxley-type neuronal models often impose limits on the scale of biologically realistic network models. However, computationally efficient Izhikevich models have been shown to qualitatively capture many experimentally observed firing-pattern types. *Hippocampome.org* is a knowledge base of neuron types in the rodent hippocampal formation, upon which we aim to build a full-scale model of the hippocampus. One of our immediate modeling goals is to create Izhikevich models that *quantitatively* reproduce various firing-pattern features of hippocampal neuron types. Previously, we developed a systematic approach to classify hippocampal neuron firing patterns and identified more than fifteen firing-pattern classes among 122 morphologically distinguished neuron types. This scheme established criteria for the classification of different firing-pattern types, such as delayed spiking, spiking with frequency adaptation, fast spiking, stuttering, and bursting. By leveraging the optimization capabilities of evolutionary algorithms (EA), we fit Izhikevich model responses to experimentally recorded and algorithmically classified firing patterns. This approach, based on EA, uses feature-based fitness functions, which also incorporate the firing-pattern class definitions. In addition, our Izhikevich models can have more than one compartment depending on dendritic invasion of neuron types across hippocampal layers. This allows layer-level specification of synaptic inputs for a neuron type in our network models. The simplified dendritic compartments are constrained based on their excitabilities and input resistances relative to the somatic compartment and directionally asymmetric spike and subthreshold signal propagation. Several of such multi-compartment models are simulated in parallel on Graphical Processing Units (GPU) in order to expedite the EA optimization process.

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Poster

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Topic: H.01. Animal Cognition and Behavior

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Title: Hippocampome.org v1.1: increasing open-access knowledge of neuron types and their properties for the rodent hippocampus

Authors: *D. W. WHEELER, C. M. WHITE, A. O. KOMENDANTOV, C. L. REES, D. J. HAMILTON, S. VENKADESH, K. MORADI, G. A. ASCOLI;
Krasnow Inst. for Advanced Study, George Mason Univ., Fairfax, VA

Abstract: Hippocampome.org is an open-access knowledge base of rodent hippocampal neurons types, each defined by the patterns of axonal and dendritic presence across the parcels of the hippocampal formation. In addition to this morphological knowledge, Hippocampome.org also includes information on biomarker expression, electrophysiological properties, and connectivity for all types. The new v1.1 release constitutes a multi-dimensional expansion of this knowledge base. Since the official 2015 launch, continued literature mining has yielded the addition of 20 new neuron types, bringing the total number to 141. Notable new content includes splitting CA1 Pyramidal cells into Superficial and Deep types, due to converging evidence of multiple distinct molecular biomarkers and electrophysiological properties, and the inclusion of Adult-Born Immature Granule cells. We also track several new properties. Neuron types are now characterized by their firing patterns, and we have identified over 20 unique firing patterns. Moreover, we have developed spiking computational models for many of the neuron types contained in the knowledge base. In the biochemical dimension, we have increased molecular-expression pieces of knowledge by 75%, by leveraging inferential evidence, which is based upon experimental observations of systematic if-then logical relationships between the presence or absence of biomarkers. Further gene expression information is derived by mining Allen Brain Atlas in situ hybridization mouse data. Additionally, we have refined the connectivity information between neuron types by expanding the evidence for known synapses (or the lack thereof) in order to confirm or, in rare cases, refute our morphologically-derived potential connectivity calculations, which are based on histological and electrophysiological evidence. Finally, we have made numerous modifications to the web portal to enhance the experience of the user, including an interactive connectivity navigator and a neuron term portal offering definitions of neuron types and properties.

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Poster

639. Learning and Memory Encoding in Hippocampal Circuits

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Topic: H.01. Animal Cognition and Behavior

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Title: Increasing biomarker information in Hippocampome.org using relational expression inferences

Authors: *C. WHITE, C. L. REES, D. W. WHEELER, D. J. HAMILTON, G. A. ASCOLI; Krasnow Inst. for Advanced Study, George Mason Univ., Fairfax, VA

Abstract: The ability to distinguish neurons based on molecular biomarker expression is a powerful research tool, and systematically linking these biochemical profiles to an established morphological characterization would aid in the investigation of neuronal circuitry. Hippocampome.org provides a web-based resource for bridging the morphological and biomarker-expression domains for neurons in the rodent hippocampal formation. Neuron types are defined in this framework based on their primary neurotransmitter and the presence or absence of axons and dendrites across the layers of subregions CA3, CA2, CA1, subiculum, entorhinal cortex, and dentate gyrus. The first release of Hippocampome.org contained 122 neuron types with specified axonal-dendritic patterns and expression information about 90 biomarkers. The ~850 available molecular pieces of knowledge (PoK) included the expression status of at least one biomarker for 81 neuron types. In the last year, we have augmented this compilation by leveraging published expression relationships between biomarkers to make inferences with calculable confidence. These take the forms of probability positive (neurons that express biomarker 1 are likely to express biomarker 2), probability negative (neurons that express biomarker 1 are likely not to express biomarker 2), and negative layer probability (neuron types with soma exclusively in a layer negative for a biomarker are likely negative). Addition of these inferential interpretations increases the PoK to ~1350 and raises the number of neuron types with information for at least one biomarker to 116 out of 122. Moreover, of the

14,884 pairwise comparisons of the biomarker expression profiles for these 122 neuron types, ~5,800 have different profiles with the inferential data, compared to ~3,600 without them.

Disclosures: C. White: None. **C.L. Rees:** None. **D.W. Wheeler:** None. **D.J. Hamilton:** None. **G.A. Ascoli:** None.

Poster

639. Learning and Memory Encoding in Hippocampal Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: Wellcome Trust and Royal Society (101208/Z/12/Z)

Title: Coordinated grid and place cell replay

Authors: *F. OLAFSDOTTIR[1], F. CARPENTER[2], C. BARRY[1];
[1]Cell and Developmental Biol., [2]Inst. of Neurol., Univ. Col. London, London, United Kingdom

Abstract: During exploration, the activity of place and grid cells represent self-location. Together these cells have been hypothesised to support spatial memory and navigation. The re-activation of place cell sequences during immobility and sleep has been proposed as a mechanism for memory consolidation. Yet the role of grid cells in replay remains unknown. We co-recorded grid and place cells while rats ran on a z-shaped track and during subsequent rest. During replay, both on the track and during rest, we found grid cells were spatially coherent with place cells. Importantly, grid-place cell replay coherence was more robust during rest and for replay events depicting place cell sequences in the original (i.e. 'forward' replay) order. Moreover, during these events the grid cell spatial representation lagged that of the place cells by approximately 10ms. Finally, grid-place cell replay coherence evolved with experience, becoming stronger as the track became more familiar. Thus, our study shows close coordination between hippocampal place cells and MEC grid cells during replay, suggesting replay may be a general property of networks encoding self-location. Moreover, these findings support the view that replay is the mechanism by which memories are consolidated to the neocortex.

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Poster

640. Learning, Remembering, and Forgetting

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Support: Kavli IRG 2015-042

Title: Measuring marmoset visual recognition memory with eye movements

Authors: *S. U. NUMMELA[1], J. T. WIXTED[2], L. R. SQUIRE[2], C. T. MILLER[2];
[1]Psychology, UCSD, LA Jolla, CA; [2]Psychology, UCSD, San Diego, CA

Abstract: The common marmoset (Callithrix jacchus) is a small bodied New World monkey with the visual specializations common to other primates including a true fovea (Mitchell and Leopold, 2015) that has the stature of a rodent making the animal model potentially powerful for the study and comparison of memory between rodents and primates. Here we present data on marmosets performing a visual preferential looking task (VPLT) that has been used to study recognition memory, the ability to perceive a recently encountered item as familiar, in humans (Wilson and Goldman-Rakic, 1994) and macaques (Jutras and Buffalo, 2010), and is known to rely on the hippocampus (Zola et al., 2000).
Trials of the VPLT were initiated by fixation of a white and black spot (1°) at the center of the display for 0.25 s, followed by an image (10°x10°). The image disappeared when the marmoset's gaze moved off of the image or after a maximum looking time of 5 s. Images were displayed in blocks of 3, 4, or 5 novel images followed by repeating their presentations in a randomized order such that an image was never immediately repeated. No reward was delivered on VPLT trials, however the marmoset was given 12 s to visually forage marmoset faces to earn reward between VPLT blocks and to ensure that gaze position remained calibrated.
Preliminary results from 3 subjects indicate a preference for novel images over familiar ones by a larger amount of time spent looking at novel images than repeated images (average of 2.6 s for novel and 2 s for familiar, $p < .0001$), but this is less separable than comparable data from macaques (2.3 s for novel and 0.8 s for familiar--from Jutras and Buffalo, 2010). This results in marmosets examining novel images for greater lengths of time than familiar images in 67% of instances ($p < .0001$). Nevertheless, this preference is substantially lower than what has been reported in macaques (90%+) and may indicate a stronger stimulus driven component to marmoset eye movement when examining visual scenes.

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Poster

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Title: Dorsal hippocampus is necessary for visual categorization in rats

Authors: *J. KIM[1], E. A. WASSERMAN[1], L. CASTRO[1], V. SLOUTSKY[2], J. H. FREEMAN[1]; [1]Psychological and Brain Sci., Univ. of Iowa, Iowa City, IA; [2]Psychology, The Ohio State Univ., Columbus, OH

Abstract: Previous studies suggested that different neural systems mediate visual categorization with stimuli that have dense and sparse defining features. The current study examined the role of the dorsal hippocampus in visual categorization of stimuli with high or low density category-relevant features in rats. Using touchscreen apparatus, we trained rats to categorize multiple abstract stimuli into two different categories. The abstract stimulus was a pentagonal configuration of five visual features. Some of the visual features were relevant for defining the category but the others were irrelevant. Two groups of rats were trained with either high or low category-relevant feature densities (3 vs. 1). Once they reached to a criterion (≥ 75%, in two consecutive days), cannula-implantation surgery was conducted into the dorsal hippocampus. After recovery, rats were given either saline or muscimol infusions into the hippocampus of different testing sessions. They were tested with the previously trained stimuli (old) as well as the different category structures in which either the category-irrelevant features were replaced with novel ones (new), the locations of the category-relevant features were relocated (relocated), or a single category-relevant feature was presented at the center of the configuration (singleton). The results showed that the task is critically dependent on intact hippocampal function. The results also showed that the rate of learning was the same for the high and low density groups. In testing, the accuracy of the density groups was equally high in both old and new conditions. However, the low density group showed more impairment in the relocated and singleton conditions than the high density group. The results suggest that the dependence on both spatial and non-spatial properties of the category-relevant features is influenced by feature density. The hippocampus is necessary for visual categorization regardless of the density of the category-relevant features.

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Poster

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BK21 plus program

Title: Differential involvement of the perirhinal cortex and postrhinal cortex in different types of visual scene memory tasks

Authors: *E.-H. PARK, J.-R. AHN, I. LEE;
Dept. of Brain & Cognitive Sci., Seoul, Korea, Republic of

Abstract: Visual scene in an animal's background plays critical roles for remembering a contextual event in episodic memory, and we have previously shown that inactivation of the hippocampus impairs such capability in rats. In the current study, we studied whether the perirhinal cortex (PER) and the postrhinal cortex (POR), the two upstream regions of the hippocampus, were differentially involved in the visual scene memory task. Rats (n=4) were trained to choose either left or right arm in association with a scene stimulus in a T-maze. Specifically, a trial started when the animal entered the stem of the T-maze and, at the same time, a visual scene stimulus (among 4 scenes; zebra pattern, pebbles, bamboo, and mountain pictures) was presented via an array of 3 LCD monitors around the T-maze. The rat was required to enter one of the arms in association with the scene to obtain food reward. Once the rat learned the task to criterion, a custom-made cannula complex equipped with guide cannulae coupled with stylets was implanted, targeting PER and POR simultaneously. Post-surgical testing showed that the rat injected with vehicle (artificial CSF) into either PER or POR was not impaired in performance. Afterward, when muscimol (MUS), a GABA-A receptor agonist, was injected in POR, severe behavioral deficits were found ($t_{(3)}$=3.73, $p<0.05$), whereas PER inactivations did not result in any behavioral deficit. Since the task required the animal to choose between different places using the visual scene stimulus, the observed deficits following the POR inactivations could be driven either from the impairment in recognizing the visual scene or from forgetting the association between the scene and spatial choice response. To further test these possibilities, a separate group of animals (n=9) were tested in a modified version of the scene memory task in

which the rat should respond differentially to a common object (sand-filled jar) in association with visual scenes. Specifically, the rat must push the jar to obtain a reward underneath the jar for one visual scene, but had to dig in the sand in the jar when the other visual scene appeared. Note that, compared to the visual scene memory task, this modified version did not require spatial choice behavior, and MUS inactivations in both PER and POR produced significant performance deficits (PER: $t_{(8)}=3.63$, $p<0.01$; POR: $t_{(6)}=4.48$, $p<0.01$). Taken together, our preliminary findings suggest that the POR plays key roles in processing visual scene memory irrespective of response types, whereas the PER may only be involved in processing visual scene memory when ambiguity associated with an object must be disambiguated using the visual scene information.

Disclosures: **E. Park:** None. **J. Ahn:** None. **I. Lee:** None.

Poster

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Title: Pattern separation in the dentate gyrus and its role in temporal lobe epilepsy

Authors: *A. MADAR[1], J. A. PFAMMATTER[1], S. RAVI[1], E. P. WALLACE[1], L. A. EWELL[2], R. MAGANTI[3], M. V. JONES[1];
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Abstract: The dentate gyrus (DG) is often seen as the gate of the hippocampus, filtering excitatory inputs from the cortex before they reach CA3. The recurrent connections of the CA3 network make it potentially prone to hyper-excitation and the breakdown of the upstream dentate gate has been shown to lead to seizures and to the development of temporal lobe epilepsy (TLE). The filtering properties of the healthy DG are thought to support pattern separation, a neural process that transforms similar input patterns of neural activity into less similar outputs, which would allow the formation of distinct memories of similar events and would prevent confusion between past and present perceptions. Accordingly, patients suffering from TLE not only experience spontaneous recurring seizures but are also often burdened by cognitive deficits such as memory impairments. Yet, no experimental study has established a relationship between TLE and the computational and cognitive roles of the DG.

We hypothesize that the development of TLE is correlated to a breakdown of the pattern separation ability of the DG network, and thus to mnemonic discrimination deficits. To test our hypothesis, we induced status epilepticus (SE) in C57Bl6 mice with intraperitoneal injections of Kainate, an established model of TLE, and compared them with control mice injected with saline. We assess their mnemonic discrimination performances with an adapted version of the "object pattern separation" task[1], based on the novelty recognition paradigm. We measure seizure frequency and severity with video/EEG recordings on each animal. Subsequently, their brains are dissected to assess the DG microcircuit and its computations. On one hemisphere, we use a novel electrophysiological assay in acute brain slices to determine the ability of the DG network to perform pattern separation on cortical activity patterns. On the other hemisphere, we employ immunohistochemistry to capture the extent of micro-circuit pathologies in DG acute slices such as mossy fiber sprouting, aberrant neurogenesis and interneuron loss.
Preliminary results indicate that non-injected wild-type (WT) mice are able to discriminate between a stationary object versus an object moved from its initial position during a first exploration. Additionally, in-slice pattern separation assay in WT mice allowed us to show that the output spike-trains of single DG output cells are less similar with each other than their driving inputs, demonstrating for the first time that the DG performs pattern separation of spike-trains measured at the level of single DG granule cells.
Ref: 1. van Hagen, B.T., et al., Behav Brain Res, 2015. 285

Disclosures: **A. Madar:** None. **J.A. Pfammatter:** None. **S. Ravi:** None. **E.P. Wallace:** None. **L.A. Ewell:** None. **R. Maganti:** None. **M.V. Jones:** None.

Poster

640. Learning, Remembering, and Forgetting

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 640.05/JJJ31

Topic: H.01. Animal Cognition and Behavior

Support: Academy of Finland grants 286384, 275954 and 284155 to MSN

Title: Hippocampal electrophysiological activity during and after trace eyeblink conditioning

Authors: *M. S. NOKIA[1], T. WASELIUS[1], I. GUREVICIENE[1], A. LIPPONEN[1], J. WIKGREN[1], H. TANILA[2], M. PENTTONEN[1];
[1]Univ. of Jyväskylä, Jyväskylä, Finland; [2]A.I.Virtanen Inst. for Mol. Sci., Univ. of Eastern Finland, Kuopio, Finland

Abstract: Hippocampus plays a crucial role in associative learning, especially when the task is complex. One such task is the trace variant of classical conditioning of the eyeblink response. Research on hippocampal electrophysiological activity related to learning trace eyeblink conditioning has mainly concentrated on analyzing local-field potentials, namely the occurrence of theta oscillations and sharp-wave ripples. Only few studies have examined the activity of single neurons and these experiments have been limited to the time of conditioning. To further study hippocampal electrophysiological activity that takes place immediately after the learning experience, we implanted adult female New Zealand White rabbits with movable silicon probes (4 x 8 channels) in the hippocampus. The rabbits were subjected to trace eyeblink conditioning using a 200-ms, 5-kHz, 75-dB tone as the conditioned stimulus and a 100-ms airpuff towards the eye as an unconditioned stimulus. The trace period was initially 500 ms. When the animal had learned to perform an adaptive conditioned eyeblink with this set-up, the trace period was lengthened to 1000 ms. Eighty conditioning trials were presented with an intertrial interval between 30 to 60 seconds. After each conditioning session, the rabbits were transferred to a cage similar to the home cage for an additional 1-hour wireless recording session. No stimuli were presented during the wireless recording and the animal was free to move around in the cage. Typically, animals would groom and actively move about the cage for some 20 minutes after which they settled down to rest. Data from both the conditioning session and the wireless recording session were analyzed for the occurrence of sharp-wave ripples, theta and single-unit spiking. The results showed robust occurrence of sharp-wave ripples and concurrent spiking of pyramidal cells during both the conditioning session and the following cage recording.

Disclosures: **M.S. Nokia:** None. **T. Waselius:** None. **I. Gureviciene:** None. **A. Lipponen:** None. **J. Wikgren:** None. **H. Tanila:** None. **M. Penttonen:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: SFB 874

MRG

IGSN

Title: Exposure to familiar and novel stimuli yields hippocampal BOLD responses for opposite contrasts depending on the duration of the habituation to the stimuli: a fMRI study in awake rats

Authors: *C. CHWIESKO[1,2], B. BOULAT[1], D. WIEDERMANN[3], M. HOEHN[3], M. SAUVAGE[2,1,4,5];
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Abstract: The specific nature of information processing in the hippocampus (HIP) and its precise mechanisms is still poorly understood. We have been the first to report HIP BOLD signal in awake rats in relation to a fMRI cognitive paradigm testing for the memory for odors. Within this frame, we showed a greater BOLD effect for 'old' odors compared to 'novel' odors (contrast old-new) in the hippocampus of rats habituated with the 'old' stimuli for 5 weeks. This pattern is consistent with some reports in humans using a similar paradigm. However, other studies also reported HIP BOLD responses for the opposite contrast (new-old). We speculated that these opposite results might stem from the use of different cognitive strategies for discriminating 'new' from 'old' stimuli during the task: either the retrieval of 'old' items or a novelty-based strategy (the detection of 'new' stimuli). In animals, extensive habituation to the odor stimuli might favor a novelty-based strategy. To test this hypothesis, we investigated HIP BOLD responses in awake rats using the same behavioral paradigm, but this time, we extended the period of habituation to the odors to 13 weeks. We used a translational human to animal fMRI compatible cognitive task, which requires to retrieve/detect 'old' and 'novel' odors. This involved an extensive training of the rats in order to minimize stress levels and maximize attention to the stimuli, as well as a head fixation surgery. In addition, new hardware such as a 40 odor- olfactometer, a head fixation system and an animal carrier had to be developed. During the experiment, a set of familiar and novel odors were presented in a block design to awake rats and hippocampal BOLD responses were assessed with fMRI. In rats, which were familiarized with the 'old' odors for 13 weeks, exposure to familiar and novel odors yielded HIP BOLD signal for the opposite contrast (new-old) than the one observed, when animals were habituated for 5 weeks to the odors (old-new). Our results suggest that the duration of the familiarization to the stimuli might indeed affect the cognitive processes at state during the task and consequently the specific type of information processed in the HIP. These findings might help explaining the discrepancy existing in the literature in humans and showed this translational approach to be a valuable tool for contributing in solving major debates in human memory research.

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Poster

640. Learning, Remembering, and Forgetting

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Program#/Poster#: 640.07/JJJ33

Topic: H.01. Animal Cognition and Behavior

Support: HHMI

Title: Internally generated retrospective activity in rat hippocampus.

Authors: *B. R. LUSTIG[1,2], Y. WANG[1], E. PASTALKOVA[1];
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Abstract: It is well known that activity in the rat hippocampus is influenced by the rats' position in space informed by external cues in the environment. However several studies have now shown that the hippocampus is able to internally generate patterned activity in the absence of changing external cues (Pastalkova 2008, MacDonald 2011 and 2013). To further our understanding of the relationship between activity in the hippocampus and memory, we have recorded internally generated activity in the hippocampus of rats while they perform a modified version of the W-Track alternation task (Frank, Brown and Wilson 2000, Kim and Frank 2009) that we are calling the three arm delayed sequence task. We found context dependent activity during the delay period was sufficient to differentiate the different arm trajectories. Furthermore in contrast to previous studies we found that analysis of correct and error trials revealed activity in the delay period was entirely retrospective. Thus internally generated hippocampal activity can reflect previous actions of animals, a signature of episodic memory.

Disclosures: B.R. Lustig: None. **Y. Wang:** None. **E. Pastalkova:** None.

Poster

640. Learning, Remembering, and Forgetting

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COFUND WHRI 2013-608765

ANR ebGlunet

Title: Functional organization of hippocampal assemblies dependent on embryonic birthdate *In vivo*

Authors: *S. REICHINNEK[1], C. HAIMERL[2], A. MALVACHE[3], R. COSSART[3];
[1]Susanne Reichinnek, Marseille, France; [2]INMED, Marseille, France; [3]Inst. de neurobiologie de la Méditerranée (INMED) - CNRS/Inserm/Aix-Marseille Univ., Marseille, France

Abstract: Cell type diversity arises during development and represents the available repertoire of neuronal behaviors which enables variable functions in different brain regions. So far, cell diversity in the hippocampus was mainly addressed in GABAergic cells. In contrast, only few studies addressed the glutamatergic CA1 pyramidal cell diversity arising from developmental and genetic parameters leading to functional and morphological differences (Graves 2012, Cembrowski 2016). We aim at understanding the relationship between the developmental temporal origin of glutamatergic neurons and their recruitment into behaviorally-relevant network dynamics and assemblies. Using an inducible genetic fate mapping approach, we are able to label neurons depending on their embryonic birth date. We use calcium imaging (GCaMP6-M) to monitor neuronal activity in head-fixed mouse voluntarily running on a cued or an un-cued treadmill using a 2-photon microscope. Monitoring hundreds of neurons in the dorsal hippocampus, we found repetitive assembly pattern and analyzed their developmental and spatial distribution during typical behaviorally relevant network oscillations e.g. theta-nested gamma and sharp wave ripples. Further on, we compare the assembly structure between minimal or location-specific external inputs. Additionally, we have probed the co-occurrence of calcium-related neuronal activity and the electrophysiological signature of the network oscillations using a contra-laterally implanted field electrode. In this way, we investigate the functional organization of neurons born at different embryonic time-points during physiological network oscillations and provide insights into how developmental origin shapes the functional organization of hippocampal microcircuits.

Disclosures: **S. Reichinnek:** None. **C. Haimerl:** None. **A. Malvache:** None. **R. Cossart:** None.

Poster

640. Learning, Remembering, and Forgetting

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SCOPE,MIC(152107008)

JSPS (16H02840, 16H01623, 16K13115)

Title: Hippocampal-prefrontal interaction during original task learning and relearning

Authors: *Y. MACHINO[1], S. TAKAHASHI[2], Y. SAKURAI[1];
[1]Lab. of Neural Information, [2]Lab. of Neural Circuitry, Grad. Sch. of Brain Science, Doshisha Univers, Kyoto, Japan

Abstract: We often recall remote memory by using the current information as a hint. This must deal with both the current information and the past information and require activities of some brain regions, e.g. hippocampus (HPC) and prefrontal cortex (PFC). There are a lot of studies reporting that these two regions are involved in memory consolidation and retrieval. A previous study showed that the correlation between HPC and PFC activities are enhanced when the rats retain memory and it is significant for the memory consolidation to send information from HPC to PFC. There is also a hypothesis that inputs from PFC to HPC are important in memory retrieval. Moreover, it is thought that HPC is engaged in comparing newer information with older information. However, it remains unclear how HPC and PFC are involved in retrieval of remote memory. Therefore we investigated neural activities of these two regions when rats were performing tasks in which they were recalling remote memory. We used two different tasks (task-A and task-B) which were interfered to each other. In both tasks, light was presented at the right or left hole randomly. In task-A, rats had to choose the right or left hole alternately regardless of the light. In task-B, they had to choose the hole at which light was presented. First, they performed task-A until they had learned it almost completely. Next, they learned task-B, and finally they performed task-A again. When they executed task-A for the second time, they presumably recalled the information about task-A that they had performed for the first time and could learn the second task-A faster. We recorded neuronal activities and LFPs from HPC and PFC simultaneously when the rats were performing task-A for the first and second times in order to explore neural mechanisms underlying retrieval of remote memory. We report the preliminary data which suggests the possibility that HPC-PFC interaction changes between original task learning and relearning.

Disclosures: **Y. Machino:** None. **S. Takahashi:** None. **Y. Sakurai:** None.

Poster

640. Learning, Remembering, and Forgetting

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Topic: H.01. Animal Cognition and Behavior

Support: The Research Council of Norway

Title: Contextual fear encoded by hippocampal single units

Authors: *L. RAGAZZI[1], A. MOLDES-ANAYA[1], V. H. BRUN[1,2], K. B. KJELSTRUP[1,2]; [1]UiT The Arctic Univ. of Norway, Tromsø, Norway; [2]Univ. Hosp. of North Norway, Tromsø, Norway

Abstract: Dorsal hippocampal neurons have spatial firing fields that contain information about the rat's location in space (O'Keefe and Dostrovsky 1971). The resolution of the spatial information gradually declines towards the ventral pole of the hippocampus (Kjelstrup et al 2008). We asked if non-spatial firing correlates are more prominent in ventral hippocampal neurons than in dorsal. To answer this question, we have used a place preference task where the emotional significance of an environment (recording arena) could be changed while leaving the visuo-spatial cues constant. Long-Evans rats were exposed to different predator odors in a custom-made environment (a 70x70cm box divided into two compartments). Odor was delivered to one of the compartments either by a closed ventilation system or by replacing the floor mat with an identical mat coated with the predator urine. Emotional significance was measured both behaviorally (e.g. avoidance and freezing) and as changes in serum corticosterone levels. Predator odor caused rats to avoid the exposure compartment when retested 3 to 48 hours later, while neutral odors did not cause any change in compartment preference. Our results show that the initial stress response during exposure and successful context conditioning was dependent on delivery method, predator species and delay interval.
We then used the same setup for recording hippocampal single units before, during and after exposure to predator odors. The odor exposure caused remapping in the dorsal hippocampus during 30 minutes of exposure to the aversive odors, but not to neutral. Preliminary results from ventral neurons showed minor changes in firing properties. These results suggest that emotional non-spatial information is encoded by hippocampal single units, consistent with the findings of Wang et al (2012 and 2015).

Disclosures: L. Ragazzi: None. **A. Moldes-Anaya:** None. **V.H. Brun:** None. **K.B. Kjelstrup:** None.

Poster

640. Learning, Remembering, and Forgetting

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Topic: H.01. Animal Cognition and Behavior

Support: NIH 1R15MH100689-01

Title: Contributions of the lateral and medial entorhinal cortex to the expression of fear memory

Authors: *B. S. EAST, JR[1], L. R. BRADY[2], J. J. QUINN[2];
[1]Neurosci., Tulane Univ., New Orleans, LA; [2]Psychology, Miami Univ., Oxford, OH

Abstract: The entorhinal cortex, with connections to the hippocampus, amygdala, and neocortex, is emerging as a critical, yet still underexplored, contributor to fear memory. Previous research looking at this structure has not only shown it plays a role in fear memory, but also that there may be heterogeneity of function among its two subregions: lateral entorhinal cortex (LEC) and medial entorhinal cortex (MEC). However, it is not well established what potentially unique roles these subregions of the entorhinal cortex serve as the literature has shown mixed results depending on target of manipulation and type of conditioning used. Furthermore, there have been relatively few studies manipulating both the LEC and MEC within the same experiment. In this experiment, Long-Evans rats were trained using either trace or delay fear conditioning. For trace conditioning, rats received 10 tone-shock pairings separated by a 28s trace interval. For delay conditioning, rats received 3 tone-shock pairings in which the tone and shock coterminated. The following day rats underwent intracranial surgery and received an NMDA-induced lesion to the LEC or MEC or received a sham surgery. After 7 days of recovery, rats were then given an 8-minute context test in the original context. The next day rats were tested for tone freezing in a novel context with three tone presentations. Finally, rats were tested for hyperactivity in an open field. Following either LEC or MEC lesions, freezing to context was significantly reduced in both trace and delay conditioned rats. Similarly, both LEC and MEC lesions appear to have produced a deficit in freezing for trace conditioned rats, but only LEC lesions produced a deficit in delay conditioned rats. Additionally, only LEC-lesioned rats showed a deficit in freezing during the trace interval. These data support the idea that the subregions of the entorhinal cortex play different roles in the expression of fear memory.

Disclosures: B.S. East: None. **L.R. Brady:** None. **J.J. Quinn:** None.

Poster

640. Learning, Remembering, and Forgetting

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Program#/Poster#: 640.12/JJJ38

Topic: H.01. Animal Cognition and Behavior

Support: HHMI

Title: Experience-dependent changes in hippocampal CA1 intracellular activity in novel and familiar environments

Authors: *J. D. COHEN, M. A. BOLSTAD, A. K. LEE;
Janelia/HHMI, Ashburn, VA

Abstract: The hippocampus is critical for converting novel experiences of places and events into long-term memories. To investigate the mechanisms underlying spatial learning, we compared intracellular features of dorsal CA1 activity in head-fixed mice exploring novel and familiar visual-based virtual reality (VR) environments. Consistent with previous extracellular studies in freely-moving rodents, new representations in VR were characterized by location-tuned place cell activity that appeared immediately upon exposure to novel environments, and also displayed higher firing rates compared to familiar environments. Unexpectedly, the novelty-induced increase in activity could not be explained by changes in either baseline (resting) membrane potential or somatic excitability. Instead, exploration of novel environments induced rapid increases in the amplitude of spatially tuned depolarizations (i.e., inputs to place fields) that drove increased spiking, high frequency bursting, and intracellular complex spike occurrence - signals which can induce synaptic plasticity. Furthermore, we observed that the subthreshold inputs across the entire extent of the environment became more spatially tuned with increasing experience (i.e., more similar across repeated traversals), reflecting learning of the new context. Thus, these signals may engage plasticity mechanisms that stabilize spatial representations for long-term storage. These findings provide a detailed description of spatial learning at the cellular level and should lead to new models of hippocampal memory formation.

Disclosures: **J.D. Cohen:** None. **M.A. Bolstad:** None. **A.K. Lee:** None.

Poster

640. Learning, Remembering, and Forgetting

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Topic: H.01. Animal Cognition and Behavior

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Title: Proximo-distal heterogeneity of hippocampal pyramidal neurons along the CA3-to-CA2 transverse axis

Authors: *Q. SUN, S. A. SIEGELBAUM;
Neurosci., Columbia Univ., New York, NY

Abstract: The hippocampal CA3 region is critical for learning and memory. Although CA3 is classically viewed as a homogeneous functional unit, recent results from genomic, behavioral and place cell recording studies have provided strong evidence for a functional segregation along the CA3-to-CA2 proximo-distal transverse axis. Here we used electrophysiological recordings and optogenetics to systemically examine the heterogeneity of intrinsic membrane excitability, mossy fiber (MF) innervation, recurrent connectivity, and direct entorhinal cortical excitation across the CA3-to-CA2 transverse axis in acute mouse hippocampal slices. First, we found a pronounced gradient of intrinsic membrane excitability along the CA3-to-CA2 transverse axis. CA3c (near the dentate gyrus (DG)) exhibits the greatest input resistance (4-5 times greater than CA2) and fires more spikes in response to constant somatic current injection, followed by CA3b, whereas CA2 has the lowest input resistance and is least excitable. The proximo-distal gradient of decreasing excitability is, in part, accounted for by an increasing gradient of Ih. Thus, CA2 expresses the largest Ih, followed by CA3a, whereas CA3c has the lowest Ih. Next, we crossed ROSA26-CAG-STOP-floxed-ChR2-EYFP with POMC-Cre mice to selectively express Channelrhodopsin2 (ChR2) in DG granule cells, which allowed us to assess the strength of MF innervation. There was a decreasing proximo-distal gradient of MF responses, with the light-evoked EPSC significantly greater in CA3c compared to CA3a. Although CA2 does exhibit a light-evoked EPSC, the response is 8-fold smaller than that observed in neighboring CA3a. Furthermore, we found that CA3 and CA2 subregions are differentially excited by the CA3 recurrent collaterals. As a result of a differential excitation to inhibition ratio, CA3b receives the greatest net excitation in response to recurrent collateral stimulation compared to the other subregions. Finally, we observed an increasing gradient of direct cortical excitation, with CA2 receiving the strongest direct input and CA3c the weakest. Taken together, our findings demonstrate distinct functional gradients of intrinsic and extrinsic properties along the CA3-to-CA2 transverse axis. The heterogeneity of intrinsic membrane excitability and functional

innervation by DG, recurrent collaterals, and entorhinal cortical input along the CA3-to-CA2 transverse axis implies that these subregions may be differentially involved in pattern separation and pattern completion during memory storage and recall.

Disclosures: Q. Sun: None. **S.A. Siegelbaum:** None.

Poster

640. Learning, Remembering, and Forgetting

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Title: Situation code in the lateral entorhinal cortex

Authors: *M. PILKIW[1], N. INSEL[1], Y. CUI[2], C. FINNEY[1], P. GURGES[1], M. D. MORRISSEY[1], K. TAKEHARA-NISHIUCHI[1];
[1]Univ. of Toronto, Toronto, ON, Canada; [2]Med. Col. of Soochow Univ., Jiangsu, China

Abstract: The entorhinal cortex is one of the brain structures thought to be responsible for the highest levels of sensory processing, representing abstract environmental information and relationships. Whereas much of neurophysiological research has focused on understanding how the medial entorhinal cortex integrates spatial information, little is known about the integration of non-spatial features of an animal's situation and the specific contribution of the lateral entorhinal cortex (LEC) in this process. We examined the coding properties of neuron populations in the LEC by recording single neuron activity while rats were trained in an associative learning paradigm under a variety of conditions. Each training session contained three epochs of 70 trials. During the first 20 trials in each epoch, a neutral stimulus (CS) was presented alone; during the subsequent 50 trials, the CS was followed by an electric stimulation to the eyelid (US), separated by a 500-ms (trace) interval. The inter-trial interval (ITI) varied between 20 and 40 s. The three epochs differed in either modality of the CS (light or sound) or conditioning chamber (i.e., spatial context), thereby allowing us to examine, within a total of six separate combinations, LEC representation of CS type, CS-US association, and the space in

which it was presented. We found that neurons not only responded to the stimuli and trace interval, but were also active during the ITI, and this activity differed between the epochs. Approximately 40% of the LEC cells showed differential firing during the ITI across all conditions, indicating selectivity for at least one of the three information types. Most of these neurons were selective for a single combination of the paradigm variables. Ensemble decoding analyses showed that the selectivity for CS modality and conditioning chamber during ITIs was stronger than that for the CS-US relationship, and this selectivity was stable for the duration of the ITI. In contrast, selectivity for the CS-US relationship was strongest during the CS and trace interval and decreased during the ITI. Selectivity for epoch was higher during ITIs that preceded trials in which the rats expressed conditioned responses (CR) compared to the trials without CR expression. This suggests that these discrete representations of past stimulus exposures in the LEC may serve as an occasion setter for an adaptive response to the upcoming CS. Our findings indicate that the LEC carries representations that depend upon the animals' history of experience in a given environment, thereby building contextual representations based on non-spatial features.

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Poster

640. Learning, Remembering, and Forgetting

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ANR-13-JSV4-0002-01

Ville de Paris

Title: Cellular network underlying enkephalin release in area CA2 of the hippocampus

Authors: ***A. FAFOURI**[1], L. THERREAU[2], V. CHEVALEYRE[2], R. PISKOROWSKI[2]; [1]Lab. de Physiologie cérébrale, équipe plasticité synaptique et réseau neu, [2]UMR 8118-CNRS/ UNIVERSITE PARIS DESCARTES, Paris, France

Abstract: The long-overlooked area CA2 is emerging as an important region for hippocampal memory formation. Area CA2 has been shown to be critical for social memory formation (Hitti and Siegelbaum 2014; Stevenson and Caldwell 2014), aggressive behavior (Pagani et al., 2014)

and appears to play an important role in detecting discrepancies between memory-driven and current sensory information converging on the hippocampus (Wintzer et al., 2014). Furthermore it has been previously reported that in area CA2, a powerful inhibitory transmission prevent CA3 inputs from driving CA2 neurons. Moreover, this highly plastic inhibition undergoes a long-term depression (iLTD) at parvalbumin-expressing (PV+) interneuron synapses and is strictly dependent on delta-opioïd receptor (DOR) activation (Nasrallah et al., 2015; Piskorowski et al., 2013). However, the cellular network required to induce the release of enkephalin, the endogenous DOR ligand, during increased neural activity is unknown. Our first goal is to determine if the source of enkephalin is intra- or extrahippocampal.
Using electrophysiology, optogenetics and confocal imaging, we are examining the cells populations that release enkephalin and determining how these cells are activated. We performed patch-clamp recordings of CA2 pyramidal neurons in acute hippocampal slices and used optogenetics to selectively evoke inhibitory post-synaptic currents (IPSCs) from PV+ interneurons. We found that the selective activation of PV+ interneurons expressing Channelrhodopsin was not sufficient to induce iLTD, suggesting that a separate population of cells is releasing enkephalin. Furthermore, using a similar strategy to selectively excite only CA3 inputs to area CA2, we found that a 10 Hz photostimulation of these axons was sufficient to evoke iLTD of inhibitory transmission. Thus, enkephalin is being released by a hippocampal source that receives excitatory input from CA3. We plan to further examine this micro-circuit and its significance for memory formation.

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Poster

640. Learning, Remembering, and Forgetting

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Topic: H.01. Animal Cognition and Behavior

Title: Experience-induced forgetting enables learning of sequential tasks.

Authors: *C. MARIOTTINI[1], L. MUNARI[1], E. GUNZEL[1], J. SECO[1], N. TZAVARAS[1], J. HANSEN[1], S. A. STERN[3], V. GAO[3], G. HODES[2], S. RUSSO[2], V. HUFF[4], M. BIRTWISTLE[1], C. ALBERINI[3], R. BLITZER[1], R. IYENGAR[1];
[1]Pharmacol. and Systems Therapeut., [2]Dept. of Neurosci., Icahn Sch. of Med. at Mt Sinai, New York, NY; [3]Ctr. for Neural Sci., New York Univ., New York, NY; [4]Dept. of genetics, MD Anderson, Houston, TX

Abstract: Remembering and forgetting are coupled phenomena both necessary to guarantee appropriate encoding and retention of experiences. We identified transcription factors (TFs) activated by stimulation that induces late LTP at Schaffer collateral (SC)-CA1 synapses in rat hippocampal slices by using a protein-DNA binding array and found among others Wilm's Tumor 1 (WT1), a transcriptional repressor also involved in mRNA translation. After reducing hippocampal WT1 activity in rats by injection of WT1 antisense, and in transgenic mice by expressing non-functional WT1, weak HFS at the SC-CA1 synapses was able to induce persistent LTP, suggesting that WT1 acts normally as a plasticity suppressor. We identified WT1-regulated genes through mRNA-seq and RT-PCR and found members of the chemokine and interleukin superfamilies, as well as IGF2, a known target of WT1. Antagonists of the CCL2 and IGF2 receptors prevented WT1 knockdown from enhancing LTP, suggesting that through the synthesis of multiple diffusible molecules WT1 controls synaptic plasticity at the circuit level. Since induction of LTP by stimulation of SC-CA1 synapses is enhanced by activity in the temporoammonic (TA) input to CA1, and hippocampal LTM requires TA input, we asked if knockdown of WT1 might facilitate LTP by freeing CA1 neurons from this dual pathway requirement. When WT1 was knocked down in CA1, activation of the TA pathway was no longer necessary for LTP induction, nor did it enhance LTP produced by SC stimulation alone, suggesting that WT1 disrupts the normal corticohippocampal circuit-level regulation of LTP. In behavioral experiments, loss of WT1 function significantly enhanced long-term memory (LTM) in two different hippocampus-dependent tasks [CFC and novel object placement (NOP)] but when trained in a two-task sequential learning protocol (NOP training, followed 48 h later by CFC training), transgenic mice showed enhanced LTM for NOP but *impaired* LTM for the subsequent CFC; however, if CFC training was delayed 10 days after NOP training, LTM for CFC was normal, supporting the hypothesis that WT1 actively suppresses plasticity for a limited period after training, thus preserving the ability of the hippocampus to encode LTM for discrete tasks. Our data indicate that the ability of the hippocampus to repeatedly encode new memories requires a period of plasticity suppression that is transcriptionally regulated, and show that WT1 plays an important role in this systems-level function. Such a complex interplay of pro- and anti-plasticity mechanisms could contribute to learning disorders, including autism and post-traumatic stress disorder.

Disclosures: **C. Mariottini:** None. **L. Munari:** None. **E. Gunzel:** None. **J. Seco:** None. **N. Tzavaras:** None. **J. Hansen:** None. **S.A. Stern:** None. **V. Gao:** None. **G. Hodes:** None. **S. Russo:** None. **V. Huff:** None. **M. Birtwistle:** None. **C. Alberini:** None. **R. Blitzer:** None. **R. Iyengar:** None.

Poster

640. Learning, Remembering, and Forgetting

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 640.17/JJJ43

Topic: H.01. Animal Cognition and Behavior

Support: Wellcome Trust Neural Dynamics PhD studentship

Title: Cholinergic modulation of DG-CA3 feedforward microcircuit dynamics and function

Authors: *L. Y. PRINCE, J. R. MELLOR;
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Abstract: Dentate gyrus granule cells provide powerful feedforward excitatory drive onto a local circuit of CA3 pyramidal cells and inhibitory interneurons, and is believed to selectively activate subsets of pyramidal cells in the CA3 recurrent network for encoding and recall of memories. Cholinergic receptors provide a key means to modulate this circuit, increasing cellular excitability and altering synaptic release, but the combined action of these changes on information processing between the dentate gyrus and CA3 remains unknown. We recorded evoked monosynaptic EPSCs and disynaptic IPSCs in CA3 pyramidal cells in response to a range of frequencies and stimulation patterns and in the presence and absence of cholinergic agonists. The short-term plasticity dynamics of these responses were used to constrain a computational model of mossy fibre driven transmission. This model was then used to analyse how a single cell model of CA3 pyramidal cells driven by constant dendritic current is perturbed by excitatory and inhibitory synaptic input. We show how the timing, frequency, and excitatory-inhibitory balance of mossy fibre input influences the activation of CA3 pyramidal by granule cell bursts, and how the presence of acetylcholine is represented in this space. We then use a spiking network model of CA3 to study encoding and recall of neuronal ensembles driven by mossy fibre input. We find that modification of mossy fibre short term plasticity by acetylcholine alters the balance between encoding and recall. This analysis provides insights into how the dynamics of mossy fibre driven activity affect the function of the CA3 network and how this is modulated by cholinergic input.

Disclosures: **L.Y. Prince:** None. **J.R. Mellor:** None.

Poster

640. Learning, Remembering, and Forgetting

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 640.18/JJJ44

Topic: H.01. Animal Cognition and Behavior

Support: NIH R15 AREA Award 1R15AG045820-01A1

Title: Validation of an adapted barnes maze for juvenile rats

Authors: *A. M. BEHSUDI[1], D. G. MCHAIL[1], N. VALIBEIGI[1], T. C. DUMAS[2];
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Abstract: The purpose of the current project is to develop a behavior task for juvenile rats that is compatible with *in vivo* electrophysiology to assess the neural bases of goal-directed spatial navigation. Most goal-directed spatial learning and memory tasks involve extended training or are performed in water mazes. Multi-day training tasks are inappropriate for developmental studies if the aim is to identify milestone ages for specific cognitive abilities. Water mazes have proven informative but, as always, are subject to contamination by stress and are not compatible with *in vivo* electrophysiology. In order to overcome these obstacles, we adapted the Barnes maze task for juvenile rats. Relative to the adult Barnes maze, the platform diameter and the number of escape holes were reduced. Long-Evans rats were exposed to the maze beginning at postnatal day (P) 17 or P22. Testing occurred across four days. On Day 1, animals were exposed to the platform in dim light without an escape box for fifteen minutes to get the animals to explore the entire platform. This phase is necessary to map hippocampal place cells prior to the learning and memory phases. On Days 2 and 3, animals were trained in bright light conditions to find a stationary escape box. On Day 4, a single probe trial was performed in bright lighting without an escape box present. We found that on Day 1, animals at both testing ages explored the entire platform. On Days 2 and 3 of testing animals of both age groups showed similar reductions in escape latencies across training blocks, with the older group being slightly quicker. On Day 4, subjects from both age groups exhibited similar dwell times near the escape location and distance to platform measures (1 Hz sampling rate). We have shown that the Barnes Maze can be adapted for use with juvenile rats. This project will allow for better understanding of the development of the hippocampus in a mammalian model by creating a goal-dependent task (by having a specific location for the rats to learn and remember) that is compatible with *in vivo* electrophysiology. Findings from the proposed studies will have implications for understanding, and perhaps even treating, brain injuries and several neurocognitive disorders including epilepsy, Alzheimer's disease, and autism spectrum disorders.

Disclosures: **A.M. Behsudi:** None. **D.G. McHail:** None. **N. Valibeigi:** None. **T.C. Dumas:** None.

Poster

640. Learning, Remembering, and Forgetting

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 640.19/JJJ45

Topic: H.01. Animal Cognition and Behavior

Support: Division of Applied Life science (BK21)

Title: Melatonin rescues memory dysfunctions, neuroinflmmation and neurodegeneration via RAGE/NF-$_K$B/JNK signaling in D-galactose-induced aging mouse model

Authors: ***T. ALI**, H. BADSHAH, T.-H. KIM, M.-O. KIM;
Biol., Gyeongsang Natl. Univ., Jinju, Korea, Republic of

Abstract: Here in, we investigated the underlying neuroprotective mechanism of melatonin against D-galactose-induced memory and synaptic dysfunction, increased reactive oxygen species (ROS), neuroinflammation and neurodegeneration. D-galactose was administered (100 mg/kg intraperitoneally (i.p)) for 60 days. After 30 days of D-galactose administration, vehicle or melatonin (10 mg/kg, i.p) was administered for 30 days. Morris water maze and Y-maze results indicated that chronic melatonin treatment attenuated D-galactose-induced memory deficits. In addition, melatonin reversed D-galactose-induced synaptic disorder via increasing the level of pre-and post synaptic protein markers. We also determined that melatonin enhances memory function in the D-galactose-treated mice possibly via reduction of elevated ROS and receptor for advanced glycation end products (RAGE). Furthermore, Western blot and morphological results showed that melatonin remarkably ameliorated D-galactose-induced neuroinflammation through inhibition of gliosis and decreasing other inflammatory mediators like p-IKKβ, p-NF-$_K$B65, COX2, NOS2, IL-1β and TNFα. Moreover, melatonin reduced the oxidative stress kinase p-JNK which reduced various apoptotic markers, i.e. cytochrome C, caspase-9, caspase-3 and PARP-1, and prevent neurodegeneration. Hence, melatonin rescued the D-galactose-induced memory/synaptic dysfunctions, neuroinflammation and neurodegeneration possibly via RAGE/NF-$_K$B/JNK signaling. Together, our data we suggest that melatonin could be a promising, safe and endogenous compatible antioxidant candidate for age associated neurodegenerative disorders.

Disclosures: **T. Ali:** None. **H. Badshah:** None. **T. Kim:** None. **M. Kim:** None.

Poster

640. Learning, Remembering, and Forgetting

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Program#/Poster#: 640.20/JJJ46

Topic: H.01. Animal Cognition and Behavior

Support: NIH MH094946 to JF

Title: Self-localization accuracy in rats is influenced by landmarks and boundaries

Authors: M. L. NGBOKOLI[1], J. FERBINTEANU[3], *J. L. KUBIE[2];
[1]Physiol. and Pharmacol., [2]Cell Biol., SUNY Downstate Med. Ctr., Brooklyn, NY; [3]Physiol. & Pharmacology; Neurol., SUNY Downstate Med. Ctr. Dept. of Cell Biol., Brooklyn, NY

Abstract: Central to the "cognitive map" hypothesis is that the collective output of hippocampal place cells provides the rat with "self-localization" information. That is, the composite firing of all place cells accurately points to a location in the environment. Using behavioral techniques we investigated rats' ability to self-localize in a large apparatus where floor marks were removed by floor cleaning and regular floor rotations.
In an earlier study we described a "spatial accuracy" task, where rats rewarded with a dropped food pellet for pausing in the region of an unmarked goal[1]. Here we describe an update, using new tracking-and-reward procedures and a much larger (6ft diameter) cylindrical chamber. Rats were pre-trained in 5-min trials to pause above a goal marker for food rewards. After a 2-hour break, the animals were tested with no marker to see if their pauses (choices) clustered near the goal location. Unmarked trials are run under reward and no-reward conditions. The goal location was changed daily. Initial results indicate that most rats learn this procedure and show a tight locus of pauses when the marker is present and a variably-tight scatter when the marker is not present. Two error measures were obtained: the distance the goal to the center of pauses (centroid), and the standard deviation of pauses from the pause center. In addition, for each trial we analyzed the paths a rat takes to approach a pause. The overall finding is that, by both measures, there is appreciable self-localization error. In addition, self-localization is more accurate near landmarks and boundaries (apparatus walls) than near the center of the chamber. Finally, on unmarked trials self localization appears to improve as the trial progresses, even without rewards.

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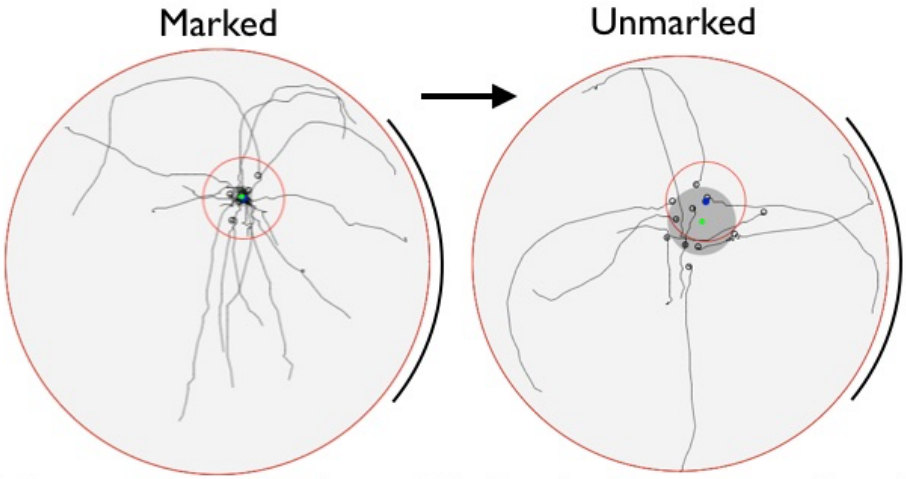
[1] Kubie,et al 2007 Behavioral Neuroscience, Vol 121(4), 751-763



Daily session pair. Red circle = goal. Small circles = pauses. green dot = choice centroid; gray = 1.0 st. dev. Lines = 4 sec paths prior to pause. black arc = white cue on wall.

Disclosures: **M.L. Ngbokoli:** None. **J. Ferbinteanu:** None. **J.L. Kubie:** None.

Poster

640. Learning, Remembering, and Forgetting

Location: Halls B-H

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Program#/Poster#: 640.21/JJJ47

Topic: H.01. Animal Cognition and Behavior

Support: NSF Grant IOS0919159

Title: Contextual latent inhibition enhances Arc protein expression in the hippocampus following testing for contextually elicited fear

Authors: ***C. D. HUDGINS**[1], T. OTTO[2];
[2]Psychology, [1]Rutgers Univ., Piscataway, NJ

Abstract: Emerging evidence suggests that the hippocampus is functionally dissociable along both its septotemporal and transverse axes. Activity-related cytoskeletal protein (Arc) has previously been shown to be tightly linked to the induction of neuronal plasticity, is expressed within hippocampal subfields, and has been implicated as a biomarker of learning. We have recently shown that trace fear conditioning enhances Arc protein levels, and that both trace conditioning and the associated learning-related enhancement of Arc can be blocked by infusing

either Arc antisense oligodeoxynucleotides (ODNs) or the NMDA receptor antagonist APV into the dorsal or ventral hippocampus prior to training. Thus while NMDAr-dependent Arc expression in both dorsal and ventral hippocampus appear to be critically involved in the acquisition of trace fear conditioning, the extent to which Arc is differentially expressed within the discrete subfields of the hippocampus following learning has yet to be fully characterized. Using traditional immunohistochemical methods, we have recently found that both exposure to a novel context and trace fear conditioning enhanced Arc expression relative to untrained control subjects; we also found that 10 days of pre-exposure to the conditioning context resulted in a marked latent inhibition of context learning, which was accompanied by a prevention of context exposure-induced Arc expression. In the present study, subjects were trained in trace fear conditioning in either a familiar (pre-exposed) context or a novel context. Arc protein expression following training was significantly higher in CA3 relative to CA1 in the dorsal hippocampus, whereas expression was notably greater in CA1 relative to CA3 in ventral hippocampus, but this enhanced expression was independent of context novelty. Interestingly, Arc protein expression following context testing in the original training context was enhanced in subjects that had been pre-exposed to that context, most notably in ventral CA1, even though these subjects demonstrated significantly less of the conditioned response. The implications for the ways in which learning is best characterized in a latent inhibition task and the extent to which Arc expression serves as a biomarker for learning and synaptic plasticity within hippocampal subregions will be discussed.

Disclosures: C.D. Hudgins: None. **T. Otto:** None.

Poster

640. Learning, Remembering, and Forgetting

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 640.22/JJJ48

Topic: H.01. Animal Cognition and Behavior

Support: CIHR

Title: Fxr1p enhances glua2 mrna translation, L-LTP and memory recall.

Authors: *E. NURO;
Ctr. for Res. in Neurosci., McGill Univ., Montreal, QC, Canada

Abstract: Proper cognitive function requires specific molecular and cellular mechanisms that allow for proper gene expression and circuitry physiology. Control of protein synthesis by mRNA binding proteins represents a major mechanism for regulating protein abundance and

site-specific localization of proteins that underlie synaptic plasticity and cognitive function. We recently discovered that a member of the Fragile X family of proteins, Fragile-X related protein (FXR1P), associates with protein synthesis machinery in neuronal dendrites and plays an important role in limiting late-phase long-term potentiation (L-LTP) and long-term memory recall. Specifically, conditional deletion of FXR1P from the postnatal forebrain enhanced hippocampal L-LTP and improved spatial memory recall (Cook, Nuro et al., Cell Reports 2014). Furthermore, loss of FXR1P increased translation of the mRNA encoding the AMPA receptor subunit GluA2. Interestingly, our findings revealed a divergence in the function of the Fragile X family of proteins whereby FXR1P represses the translation of GluA2 via its 5'UTR, FXR2P promotes the translation of GluA2 mRNA via its 5' and 3'UTR, while FMRP has no effect. We are currently following up on these results to further dissect the molecular mechanisms involved. Furthermore, using *in vivo* tools such as *in utero* electroporation in mice, we are studying the *vivo* localization of FXR1P in CA1 pyramidal cells of the hippocampus and its relationship to GluA2 protein at excitatory synapses. Future studies will also determine if and how FXR1P may be related to CNS disorders associated with cognitive and intellectual disability.

Disclosures: E. Nuro: None.

Poster

640. Learning, Remembering, and Forgetting

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: DBTO/BCN/BJ/0402

DSTO/BCN/BJ/1102

DSTO/BCN/BJ/1297

JTT/MUM/INST/IIOS/201314/ 0033

Title: Acquisition dependent influence of mental schema on problem solving in mice

Authors: *V. SINGH[1], R. BHATT[1], S. KUNDU[1], S. SHRIDHAR[1], A. SINGH[1], S. SAM[2], B. JAYAPRAKASH[1];
[1]Ctr. for Neurosci., [2]Ctr. for Nano science and Engin., Indian Inst. of Sci., Bangalore, India

Abstract: Memories of related events and facts are stored in form of neocortical associative networks (mental Schema) and they help in rapid consolidation of new memories that are similar

in character. Although neocortex is shown to be involved, it is not clear if this is true for learning larger sets of information with content richness similar to the initial schema. In order to address this we trained animals to learn same sets of flavour place associations(paired associates) in three different ways viz., i) Solitary Learning: Two sets of association are presented independently one after the other ii) Relational Learning: Second set is presented in relation to first set and iii) Incremental Learning: The paired associates are presented in small steps. We find that only the animals that underwent relational learning were able to acquire both set of associations. Both the solitary group as well as the incremental group were unable to learn all the associations in its entirety. Comparing the three groups we show that older memories help in acquisition of new memories only if presented in a relational manner. We hypothesise and show that these interactions amongst memories can bias the animal's ability to solve problems derived from prior knowledge.

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Poster

640. Learning, Remembering, and Forgetting

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 640.24/JJJ50

Topic: H.01. Animal Cognition and Behavior

Support: RIKEN

Title: Direct mutual connectivity between hippocampal CA1 and the superficial layers of the entorhinal cortex

Authors: ***J. SUH**[1,3], M. MARIOUTINA[1,3], I. WICKERSHAM[2], S. TONEGAWA[1,3,4,5]; [1]Picower Inst. Learning & Memory, [2]MIT, Cambridge, MA; [3]RIKEN-MIT Ctr. for Neural Circuit Genet., Cambridge, MA; [4]RIKEN Brain Sci. Inst., Wako, Japan; [5]Howard Hughes Med. Inst., Cambridge, MA

Abstract: The entorhinal cortex (EC) is a major input and output structure of the hippocampal formation, entertaining the role of the nodal point of cortico-hippocampal circuits. The findings of the grid cells in all layers of the EC and recent studies on the functions of EC inputs to the hippocampal CA1 indicate that each layer of the EC play a unique role in spatial representation and episodic memory processing. However, it is still unknown that how the grid formation is generated and the position of an animal is computed within the EC circuits. Furthermore, due to technical limitations, we have a limited understanding of whether or how the different sources of

inputs to EC layers are distributed with different strengths on cells in each layer, which may be essential for a mechanistic understanding of functional circuit operations in the EC. To map an intrinsic organization between EC layers and identify specific sets of inputs to these layers, we employed genetically modified and monosynaptically restricted rabies tracing methods in combination with multiple transgenic mouse lines in which Cre recombinase expression is confined to a specific layer of the EC. We found that superficial layers (II-island and III) in dorsal EC receive a similar pattern of inputs from the anterior dorsal thalamus and subcortical neuromodulatory systems including cholinergic projections from the medial septum and serotoninergic inputs from the median raphe nucleus. Furthermore, within the entorhinal-hippocampal circuits, we found that EC layer III receives extensive inputs from entire CA1, whereas layer II receives inputs from proximal CA1 and CA2. These findings are contradicting the notion that CA1 pyramidal cells mainly connect to deeper layer (V) of the EC, which subsequently connects to upper layers (II and III) of the EC. These differences probably reflect different functions of these cortical layers in mediating spatial and temporal information processes. Our results will provide a foundation of further functional dissection of the entorhinal-hippocampal circuits.

Disclosures: J. Suh: None. **M. Marioutina:** None. **I. Wickersham:** None. **S. Tonegawa:** None.

Poster

640. Learning, Remembering, and Forgetting

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 640.25/JJJ51

Topic: H.01. Animal Cognition and Behavior

Support: Sandia National Laboratories' LDRD Grant

Title: Formalizing function within the hippocampal trisynaptic circuit

Authors: *W. SEVERA, O. PAREKH, C. JAMES, J. B. AIMONE;
Sandia Natl. Labs., Albuquerque, NM

Abstract: Claims regarding the functions of regions within the hippocampal formation are touted without clear definitions or experimental justification. For example, it is often taken for granted that the Dentate Gyrus (DG) performs pattern separation; the CA3 is auto-associative memory; and the CA1 forms a comparator. However, there are few mathematically rigorous models supporting the methods by which these functions are accomplished, and these theoretical frameworks often fail to fully explain experimental observations of the hippocampus, such as the

presence of adult-born granule cells in the DG or the dynamics of place cell ensembles in the CA3 and CA1. One of the affirmative examples is the formal modular code used by Fiete and colleagues to describe grid cell activity in mEC, one of the principle input regions to the hippocampus. The development of the equivalent for hippocampal regions like the DG and CA3 may provide a stronger theoretical footing upon which we can frame experimental results, and here, we introduce mathematically rigorous frameworks for these two areas. Our DG model provides provable pattern separation via a closed form, static sparse coding. We begin with an abstract formulation and generalize to a more advanced model. This allows us to incorporate constrained input patterns and mixed coding reflecting adult neurogenesis. We also discuss a method to formally embed more conventional DG models within our framework. For CA3, a region often approximated as a straightforward Hopfield-like attractor network, we instead consider a dynamical approach leveraging non-stationary limit cycles. Specifically, we formalize CA3 as a family of dynamical systems where information is coded in stable, non-fixed-point orbits. One key advantage of this approach is that it provides a mechanism by which contextual inputs can gracefully change the way in which memories are formed and retrieved. Only one system is expressed in any given context, but the concept of neuromodulation provides a method for global transitions between these systems via topological conjugacy. While these mathematical frameworks for considering the hippocampus do not challenge the conventional wisdom regarding high level hippocampal function, they seek to formalize the processes through heretofore never considered mechanisms.

Disclosures: **W. Severa:** None. **O. Parekh:** None. **C. James:** None. **J.B. Aimone:** None.

Poster

640. Learning, Remembering, and Forgetting

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 640.26/JJJ52

Topic: H.01. Animal Cognition and Behavior

Title: Neuronal plasticity of dorsal hippocampus in rats subjected to cognitive training: female and male rats

Authors: L. VILLANUEVA ESPINO[1], *A. B. SILVA[2];
[1]Lab. de Neurofisiología Exptl., Escuela de Biología, Benemérita Univ. Autónoma de Puebla, Puebla, Mexico; [2]Benemérita Univ. Autónoma de Puebla, Puebla, Mexico

Abstract: Several lines of research have established that the hippocampus is involved in learning and memory processes, and these processes are developed through structural and physiological changes of the neural systems. Morphological changes in the brain in response to cognitive

training and environmental stimulation have been mainly obtained from male rats. According to this, the aim of this study was to determine if there are sexual dimorphism in neuronal plasticity of dorsal hippocampus after cognitive activity. Long Evans female and male rats (n=8 per sex) were used to perform four cognitive tests, which were locomotor activity in open field, Morris water maze, Barnes circular maze and object recognition task. In order to observe the effects of the cognitive tests, we compared the morphology of the apical and basolateral dendritic arborization, as well as density of dendritic spines of CA1 and CA3 regions of the dorsal hippocampus of rats that performed the tests vs. control rats. The dendritic morphology was measured using the Golgi-Cox procedure followed by a Sholl analysis. Both, female rats and male rats performed similarly in all tests, but female rats were hiperactive in locomotor activity. The morphology analysis, showed increases in the apical arborization of CA1 neurons, and increases in the density of dendritic spines of the apical and basolateral arborization of CA1 neurons and CA3 by cognitive activity effect. These results can be related with the patterns of connection that receives each neuronal type and the role played by each structure in the processing of the information. The performance of cognitive tests favors the increment in the dendritic arborization of CA1 region as well as the increase in the density of dendritic spines of CA1 and CA3 hippocampal regions without showing signs of sexual dimorphism.

Disclosures: L. Villanueva Espino: None. **A.B. Silva:** None.

Poster

640. Learning, Remembering, and Forgetting

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Program#/Poster#: 640.27/JJJ53

Topic: H.01. Animal Cognition and Behavior

Support: NIAAA Grant 1P50-AA022534-01

Title: Alterations in touchscreen pattern separation following moderate prenatal alcohol exposure in the mouse

Authors: *J. A. KENTON, JR[1], M. JOSEY[1], B. J. CLARK[1], J. L. BRIGMAN[1,2];
[1]Univ. of New Mexico, Albuquerque, NM; [2]UNM Hlth. Sci. Ctr., New Mexico Alcohol Res. Ctr., Albuquerque, NM

Abstract: It is well established that exposure to alcohol during development leads to alterations in neurogenesis and deficits in hippocampal-dependent learning and memory. Recent evidence suggests that even more moderate alcohol consumption during pregnancy can have negative impacts on the cognitive function of offspring in the absence of hallmark morphological defects.

However, methods for assessing impairments differ greatly across species, making it difficult to translate preclinical findings into potential therapeutic avenues. We have recently demonstrated the utility of a touch-screen operant measure for assessing hippocampal function in mice. Here, we integrated a well-established 'drinking in the dark' exposure model that produces reliable, but more moderate, levels of maternal intoxication (daily dam BAC ~85 mg/dL) with a touch-screen based trial unique, delayed nonmatching-to-location (TUNL) task to examine the effects of PAE on hippocampal-sensitive behavior directly analogous to those used in clinical assessment. Briefly, PAE and SAC offspring mice were trained to touch a single visual stimulus ("sample phase") in one of 10 possible spatial locations (2x5 grid) in a touch-screen operant system. After a delay, animals were simultaneously presented with the original stimulus and a rewarded stimulus in a novel location ("choice phase"). Separate cohorts of PAE and saccharin (SAC) control mice were trained on different distinct progressions of difficulty. We found that PAE mice were able to attain performance similar to SAC control mice at progressively more difficult separations. In addition, training initially on decreased separations and then progressing to farther separations revealed differences in performance by PAE mice. Current studies are investigating alterations in neurogenesis in PAE that may drive deficits seen on the TUNL task and examining online neuronal correlates of behavior during distinct phases of pattern separation learning.

Disclosures: **J.A. Kenton:** None. **M. Josey:** None. **B.J. Clark:** None. **J.L. Brigman:** None.

Poster

640. Learning, Remembering, and Forgetting

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 640.28/JJJ54

Topic: H.01. Animal Cognition and Behavior

Support: Northern Norwegian Regional Health Authority

Title: Intrahippocampal growth hormone modulates spatial memory

Authors: ***K. G. HAUGLAND**[1], A. MOLDES-ANAYA[1], K. B. KJELSTRUP[1,2], V. H. BRUN[1,2];
[1]UiT The Arctic Univ. of Norway, Tromsø, Norway; [2]Univ. Hosp. of North Norway, Tromsø, Norway

Abstract: Growth hormone (GH) is a neuromodulator involved in cognitive deficits of ageing and endocrine disturbances (Nyberg and Hallberg 2013). Several studies have shown that systemic elevation of ghrelin or GH is beneficial for learning and memory (e.g. Diano et al.,

2006), however the main target areas of the brain and mechanisms of action are yet to be determined. We have altered GH levels selectively in the hippocampus of male Long Evans rats and investigated the effects on memory performance. The dorsal hippocampus was transfected with recombinant adeno-associated virus (AAV) expressing either GH, GH-antagonist (GHA) or green fluorescent protein (GFP) only. These animals with chronic intrahippocampal GH overload or deficit were subsequently tested in hippocampus-dependent memory tasks, such as the water maze and object displacement tasks. Our findings show that the GH levels affected memory in a dose-dependent manner. Preliminary results indicate that a proportion of the animals expressing high GH levels exhibited impaired spatial memory in the water maze task. Histological analysis revealed cell death in the transfected areas of some animals, probably caused by hyperexcitation in the affected neurons. In contrast, animals with no tissue damage, but still increased GH levels, displayed significantly better memory in an object displacement task. Furthermore, the memory performance of rats with high levels of GHA did not differ significantly from the control rats. To conclude, these preliminary results indicate that GH levels in the hippocampus are important for plasticity and modulate spatial memory performance in rats. Very high levels of GH may lead to hyperexcitation and eventually cell death, consistent with the view that GH facilitates long-term potentiation by increasing synaptic density. A slightly elevated GH level in dorsal hippocampus may improve cognitive performance and memory.

Disclosures: **K.G. Haugland:** None. **A. Moldes-Anaya:** None. **K.B. Kjelstrup:** None. **V.H. Brun:** None.

Poster

640. Learning, Remembering, and Forgetting

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 640.29/JJJ55

Topic: H.01. Animal Cognition and Behavior

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Ellison Medical Foundation New Scholar in Aging

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Title: The contribution of hippocampal oxytocin receptors to social memory processing

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Abstract: The trisynaptic hippocampal pathway dentate gyrus (DG) DG-CA3-CA1 plays a critical role in processing contextual information and object recognition. Recent studies suggest a role for the CA2/3 subregions in processing social memories, raising the question of whether and how the same hippocampal sub-regions process social and object information. Here, we used optogenetic terminal specific silencing and identified roles for distinct CA2/3 outputs in discrimination of objects and social stimuli. This double dissociation suggests that CA2/3 segregates social and object information to appropriate downstream targets through distinct neural pathways. Based on the enrichment of receptors for the social hormone oxytocin (Oxytocin receptors, Oxtr) in the CA2/3 subregions and dorsolateral septum but not in CA1, we hypothesized that oxytocin signaling in CA2/3 plays a critical role in processing social memories. In order to determine the necessity of oxytocin receptor signaling in CA2/3 in social memory, we injected AAV Cre recombinase into the CA2/3 subregion of Oxtr conditional knockout mice. Knocking out oxytocin receptors in CA2/3 produced a strong deficit in social memory processing, with no effect on object recognition. We are currently investigating the neural mechanisms by which Oxtr signaling in CA3 impacts social memory processing. Together, these studies begin to illuminate how an ancient neuromodulatory hormone, oxytocin, utilizes a basic memory processing circuit scaffold in the hippocampus to guide social behavior.

Disclosures: T. Raam: None. **A. Besnard:** None. **K. McAvoy:** None. **A. Veenema:** None. **A. Sahay:** None.

Poster

641. Learning and Memory: Sensory and Association Cortex

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Natural Science Foundation China (81471123)

Title: Synapse formation and memory cell recruitment for associative memory

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Abstract: Associative learning and memory are essential for the cognitions. The structural and functional plasticity of the synapses and neurons are presumably associated to learning. It remains unclear about the natures and recruitment of associative memory cells that accept, storage and retrieve multiple signals. We studied this issue in mouse model of associative memory by multidisciplinary approaches *in vivo*. Pairing stimulations to whiskers and olfaction led to reciprocal cross-modal reflexes, i.e., odorant-induced whisker motion and whisker-induced olfaction response. The cross-modal reflexes were also induced by the co-activation of barrel, piriform and S1-tail cortices. In the mice that express the cross-modal reflexes, their barrel, piriform and S1-tail cortices mutually innervated by the new synapses formed from their projected axons to target cells. A substantial portion of the neurons and astrocytes in each of these cortical areas became to encode the newly acquired signals alongside the innate signal. Some of these associative memory cells expressed the different activity patterns in response to the acquired signals and innate signal, and others encoded similar pattern in response to these signals. These associative memory cells in each of these cortical regions received the synaptic inputs from the newly established afferents alongside from the innate afferents. The antagomirs of miRNA-324p/miRNA-133a attenuated the formation of associative memory, the formation of new synaptic innervation and the recruitment of associative memory cells. Thus, the co-activation of the sensory cortices initiates their mutual synaptic innervations, which drive their target neurons to be recruited as associative memory cells that encode the new learnt signals (cross-modal memory) alongside the innate signal (native-modal memory). In terms of the storage and retrieval of the associated signals, the natures of associative memory cells include the followings. They are recruited to encode multiple signals being associated. They receive multiple synaptic inputs from the cortices of signals' origins. Their axons project toward the brain areas being associatively activated, as well as other brain areas for memory presentation. Their recruitments are downregulated by altering the gene and protein expressions. The upregulation or downregulation of these neurons, their synaptic inputs and axon projections alters memory capacity. Their axons project to the contralateral cortices and make the synapse innervations that send the acquired signals for unilateral learning toward bilateral memory.

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Poster

641. Learning and Memory: Sensory and Association Cortex

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Title: Unconsciously implanted memory reflected in behavioral test

Authors: ***X. ZHENG**[1], J. HE[1], Z. ZHANG[1], X. CHEN[1], W. SUN[1], X. LI[1], Y. GUO[2], G. NG[4], P. TANG[5], D. LU[1], H. FENG[1], Z. ZHANG[3], F. XU[3], Q. LIU[3], Z. WANG[6];
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Abstract: Recent studies have shown that the entorhinal cortex switched neocortical plasticity and encoding of associative memory in the auditory cortex likely through cholecystokinin (CCK) projection neurons. In the present study we hypothesize that associative memory could be implanted in the anesthetized animal's brain. A bilaterally electrode-implanted rat was trained to retrieve water-reward from either the left or the right hole depending on which hemisphere of the auditory cortex stimulation was triggered after it initiated the trial. After the stimulation site of one hemisphere was infused with CCK, a previously irrelevant light stimulus was then paired with the electrical stimulation of the infused hemisphere for multiple sessions in the anesthetized rat. The auditory cortex neurons responded to the light stimulus in both anesthetized and behavioral conditions. All 6 rats approached to the "designed" hole after they triggered light stimulus instead of electrical stimulation of the auditory cortex one week after the first conditioning. We further hypothesized that the high-frequency (HF) stimulation is required to induce release of CCK that enables the encoding of visuo-auditory associative memory. With implanted stimulation electrode and optical fiber in the auditory cortex and injected AAV-DIO-eYFP-ChR2 in the entorhinal cortex in the CCK-CRE mouse, we conditioned the electrical stimulation of the auditory cortex with footshock. The mouse showed no freezing response when the light stimulus was presented. We then anesthetized the mouse, used HF laser stimulation in the auditory cortex of the cortical projection fiber of the entorhinal CCK neurons and then paired the light stimulus and the electrical stimulation of the auditory cortex with low frequency. The mouse changed to freeze when the light was presented after the above pairing, indicating that the mouse associated the light stimulus with the ES, and recalled the aversive memory. The

behavioral experiments revealed that the artificially memory was transferred to the behavioral action, providing a scientific foundation for “memory implantation”.

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Poster

641. Learning and Memory: Sensory and Association Cortex

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Support: Russian Ministry of Education and Science grant 14.607.21.0092

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RFBR Grant 13-04-01892

Title: Encoding of fear memory about complex stimulus in the mouse parietal association cortex: c-fos two-photon imaging

Authors: ***M. ROSHCHINA**[1], O. IVASHKINA[1], K. TOROPOVA[1], A. KALASHNIKOV[2], A. GRUZDEVA[1], K. ANOKHIN[1];
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Abstract: In natural environment associative memory often integrate several stimuli from different sensory modalities. Previously we have developed behavioral paradigm for fear conditioning using multisensory compound stimulus that mimics complexity of natural learning. In the present study, we examined encoding of fear memory about compound stimulus by neurons of the mouse parietal cortex. We trained transgenic c-Fos-EGFP mice in a fear-conditioning task to a compound stimulus (tone + light). A week later, we performed three retrieval sessions using separate sound or light as well as the compound stimulus. To monitor neuronal activation, we performed two-photon in vivo imaging of EGFP expression in the parietal associative cortex. Based on the intensity of fluorescence, all neurons were divided into the two groups: highly- and weakly-active. Number of highly-active neurons were increased in trained mice compared to the control mice in all retrieval sessions, while the number of weakly-active neurons decreased in all the sessions. Next, we analyzed neurons that showed high fos-EGFP expression only in one of the retrieval session. We found three activation specificities of

such neurons: light-related, sound-related and compound CS-related neurons. The number of light-dependent highly-active neurons increased in the trained mice compared to the control mice. The number of sound-dependent and compound CS-dependent neurons was equal in the trained and control mice. Also with the calcium imaging we shown that neurons active during learning later respond to tone and light during retrieval sessions. Then we used c-fos immunohistochemistry to analyze activation pattern in different brain areas after optogenetic stimulation of parietal cortex. Taken together our data suggests that coding of complex associative memory in the parietal cortex involves at least three neuronal assemblies with different response specificity to the components of the compound CS.

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Poster

641. Learning and Memory: Sensory and Association Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: Russian Ministry of Education and Science grant 14.607.21.0117

Title: Differential involvement of areas and layers of the mouse neocortex in associative fear memory formation and retrieval

Authors: *K. A. TOROPOVA, T. A. KUNITSYNA, O. I. IVASHKINA, M. A. ROSHCHINA, K. V. ANOKHIN;
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Abstract: Associative learning is a fundamental mechanism for experience-dependent cortical modification. Though synaptic aspects of this process has been thoroughly studied, less is known about how it is implemented at the level of cortical circuits. Predictive coding theories suggest that classical conditioning leads to formation of cortical representations that differentially affect supragranular (L2/3) and infragranular (L5/6) cortical layers. While deep layers of the sensory cortex mediate top-down predictions from anticipatory model generated by frontal areas, superficial layers convey bottom-up prediction error signals that update prior representation according to sensory input. To study these processes we used auditory fear conditioning and c-Fos neuroimaging of layer-specific activation of different associative (infralimbic, prelimbic, cingular, retrosplenial, frontal associative and parietal associative) and sensory (primary auditory, ventral and dorsal areas of the secondary auditory) cortices after memory acquisition

and retrieval in mice. We found that presentation of auditory CS and footshock during training leads to activation of cingulate cortex and the ventral part of secondary auditory cortex. Retrieval of associative memory about CS a day later produces preferential activation of cingulate, prelimbic, infralimbic and parietal associative cortices. This activation was specific in L2/3, L5 and L6 of cingulate cortex and L5 and L6 of prelimbic cortex. Layer-specific analysis of the primary auditory cortex showed that memory acquisition involved proportionally more L2/3 neurons, while CS presentation during retrieval involved proportionally more L5 neurons. Thus, our data suggest that cued conditioning can lead to shift from activation of supragranular layers during acquisition phase to infragranular layers during retrieval phase in the primary auditory cortex and overall preferential activation of associative areas during memory retrieval versus sensory cortex activation during memory acquisition.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: RSCF #14-15-00685

Title: Pavlovian engram conditioning: properties and imaging of neural circuitry

Authors: *K. ANOKHIN, N. VOROBYEVA, K. TOROPOVA, O. IVASHKINA;
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Abstract: Pavlov's original work on classical conditioning used reinforcement of immediately present environmental event. An important fundamental question is whether an engram of a distant past event can be also conditioned. Though there are evidence that this can happen if an engram is ecphorised in the presence of reinforcing stimulus, surprisingly little work has been done on the specific properties of such association and its neural mechanisms. Here we used a model of such engram conditioning, the context preexposure facilitation effect paradigm (Fanselow, 1990; Rudy and O'Reilly 2001) to explore associability of activated engram with a footshock reinforcement in mice, specificity of such associative memory, its durability and neural activity at its retrieval. Mice were first allowed to explore a new context A for 5 min and then at different delays received a 2 sec footshock immediately after being replaced in this context. We showed that the contextual engram in mice can be fear conditioned in the interval

from 30 min to at least 30 days between its acquisition and retrieval. Resulting association persisted for at least 30 days after such engram conditioning. The fear for the context A was specific, it did not develop if immediate shock was delivered in a context B and there was no freezing in a novel context C. We next studied how engram conditioning changes patterns of brain activity during its subsequent retrieval. For this we compared c-Fos expression induced by exploration of context A in mice from conditioned and unconditioned groups. Both groups explored the context 6 days before being tested, but the conditioned group also received immediate footshock in this context 3 days before testing. Expression of c-Fos was significantly elevated in the conditioned engram group in the areas of associative neocortex (frontal associative cortex, prelimbic and infralimbic cortex, cingular, retrosplenial and parietal cortex), in central and lateral amydgala and in CA1 region of hippocampus. Interestingly, dentate gyrus and CA3 region had similar c-Fos activation in the conditioned and unconditioned engram groups. We also performed c-Fos TRAP combined with c-Fos imaging to reveal cellular overlap in the episodes of context engram acquisition and its conditioning in the same animals. Our data suggest that engram acquisition and its conditioning though often occurring together, are distinct processes that can be isolated and studied separately.

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Poster

641. Learning and Memory: Sensory and Association Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: RSCF Grant 14-15-00685

Title: Associative fear memory to a compound cue in mice: behavioral and neuronal features

Authors: *O. I. IVASHKINA, K. TOROPOVA, M. ROSHCHINA, K. ANOKHIN;
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Abstract: Though cellular mechanisms of associative memory are commonly studied in Pavlovian fear conditioning (FC) to a single discrete stimulus, natural learning often involves complex stimuli composed of several sensory modalities. To study neural mechanisms of such learning we used FC to a compound cue (CC) consisted of auditory (tone, T) and visual (blinking light, L) stimuli. First, we showed that memory about CC and its separate components mature over different time after training: memories for CC and tone were manifested in freezing

behavior at all times tested starting from the first day after the training, whereas light component produced significant freezing response only starting from 3 days after the training. Next, we showed the same dissociation of memories in extinction experiments. Extinction of the one component of CC memory 1 day after the training did not affect memory about another component or CC. Extinction of the one component of CC memory 7 days after training resulted in extinction of memory about another component or CC. Finally, we performed c-Fos imaging of cellular activity in number of brain regions after formation and retrieval of memory for CC and its separate components. We showed that learning the CC resulted in significant activation of prelimbic cortex, lateral and central amygdala. Retrieval of memory of the CC and its separate components 7 days after the training resulted in significant activation of prelimbic and parietal association cortices, whereas basolateral amygdala was activated only after CC memory retrieval. Moreover we compared patterns of c-Fos expression after 1 and 7 days retrieval. Finally, we performed in vivo two-photon imaging of retrieval-induced c-Fos expression in the parietal cortex of fos-EGFP transgenic mice and found that there are at least three neuronal populations with different response specificity to the compound signal and its components. Taken together, our data suggests that complex stimuli can establish three different neuronal representations that can be used separately in the behavior.

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Poster

641. Learning and Memory: Sensory and Association Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: Russian Ministry of Education and Science grant №14.607.21.0092

Title: Comparison of neuronal activity in the mouse parietal associative cortex during fear memory formation and retrieval: *In vivo* imaging by immediate early gene expression

Authors: *A. GRUZDEVA, O. IVASHKINA, M. ROSHCHINA, K. ANOKHIN;
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Abstract: Memories are hypothesized to be encoded by distributed cell assemblies, however little is known about consistency of activation of such neuronal populations. An essential question is how similar are these activations during memory encoding and subsequent episodes of retrieval. To study this question we investigated neuronal activity of the mouse associative

cortex in auditory fear conditioning and memory recall in response to conditioned sound. We used optic fiber recording and two-photon visualization of activated neurons in the cortex of awake transgenic mice in which the expression of enhanced green fluorescent protein (EGFP) was under the control of *c-fos* immediate early gene promoter. With optic fiber technique we characterized time course of induced fos-EGFP expression. Then we compared populations of active neurons in the same area of parietal associative cortex before learning, following learning and following memory retrieval. The largest proportion of identified neurons was active during all three conditions (79%). Most neurons, which were not active before learning, but were activated during learning, were also activated during memory retrieval (10% of all identified neurons). The small parts of identified neurons were activated only during learning or memory recall (3% or 2% respectively). Thus, our results indicate that the main part of neuronal population in the mouse parietal associative cortex that was transcriptionally activated during fear memory encoding, was then reactivated during cue-induced memory retrieval.

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Poster

641. Learning and Memory: Sensory and Association Cortex

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Topic: H.01. Animal Cognition and Behavior

Title: Long-term episodic-like memory in object recognition model in three wild rodent species

Authors: *D. IVASHKIN, O. IVASHKINA, K. TOROPOVA, K. ANOKHIN;
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Abstract: One of the key features of human memory is the ability to recall the entire complex episode in terms of what, where and when it happened. For a long time, it was assumed that episodic form of memory exists only in humans. Animals cannot give a verbal report about a personal past experience, yet episodic-like memory can be demonstrated as an integrated behavioral response for “what”, “where” and “when” questions. Dere et al. in 2005 proposed model of episodic-like memory for rodents based on the recognition of new neutral objects. This model has been used only to test the short-term memory. However, episodic memory in humans can be stored for years. Therefore, an important question is whether rodents are able to form long-term episodic-like memories in such a model. To address this problem, we used the modified version of task proposed by Dere et al. in wild rodent species: wood mice (Sylvaemus uralensis), bank voles (Clethrionomys glareolus) and field mice (Apodemus agrarius). Each

rodent received 5 experimental sessions (10 min each). During two pretraining sessions (PTS) animals explored new empty arena (PTS 1) or the same arena with 2 new objects (PTS 2, 6 h later). On training session (TS) 1 (16-18 h after the PTS 2), 4 novel objects were presented arranged in a triangle spatial configuration. On TS 2 (8 h later), another 4 novel objects were presented in a different spatial arrangement (in 4 arena corners). During the long-term memory test session (16 h after the TS 2) 4 objects were presented: old stationary (object from the TS 1 on the same position), old displaced (object from the TS 1 on the new position) and two recent objects from the TS 2 on the same positions. The duration of the active interaction with objects by animals was measured during all sessions. The “what”, “where” and “when” ratios were calculated as proportion of interaction with different types of objects. We demonstrated that duration of active interaction with objects in all rodent species increased from the first to the last experimental session, while the total exploratory behavior in the arena decreased. The presence of long-term episodic-like memory as a behavioral response to each of “what”, "where" and "when" questions was revealed only in field mice. Wood mice and bank voles were able to recall the memory only about the “where” component. We also showed that the individual variability of the exploratory behavior in bank voles and wood mice was significantly higher compared to the field mice. Thus, in this study we demonstrated the long-term episodic-like memory about the neutral event in wild rodents. The ability to form such memories may be important for the adaptive behavior of animals under the natural conditions.

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Poster

641. Learning and Memory: Sensory and Association Cortex

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Title: Roles for state-specific corticothalamic and thalamocortical communication in visual system plasticity

Authors: *J. DURKIN[1], A. SURESH[5], Q. SKILLING[2], J. COLBATH[3], M. ZOCHOWSKI[4], S. J. ATON[3];
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Abstract: Orientation Specific Response Potentiation (OSRP) is a form of plasticity in primary visual cortex (V1), which is initiated by waking visual experience and dependent on subsequent sleep. OSRP consolidation is accompanied by an increase in V1 neuron firing following visual stimulus presentation, and is correlated with changes in spike-field coherence during Non-Rapid Eye Movement (NREM) sleep. Thus, we hypothesized that state-specific features of thalamocortical communication, such as NREM-specific oscillatory activity, are crucial for OSRP consolidation. To address this hypothesis, we performed two experiments using a combination of stereotrode recordings and state-specific optogenetic manipulations in mice. These experiments target distinct parts of the visual circuit, and address the necessity of both synchronization of thalamocortical activity, and thalamic (LGN) input to V1 for OSRP consolidation. For both sets of experiments, we measured OSRP across a 12-h interval following experience, the amplitude and coherence of thalamocortical oscillatory activity, and functional interactions between neurons in thalamus and cortex. We find that optogenetic inhibition of corticothalamic feedback during NREM sleep, but not Rapid Eye Movement (REM) sleep or waking, blocks OSRP. Our data suggests that synchronous activity between cortex and thalamus during NREM is necessary for OSRP consolidation. Preliminary data from LGN thalamocortical inhibition experiments indicate thalamocortical activity during REM may also play a role in OSRP. These studies will enhance understanding of both structure- and state-specific properties underlying a novel form of cortical plasticity.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: Junior Faculty Development Program

Title: Impact of visual experience on oscillatory state and visual recognition memory in primary visual cortex

Authors: *R. KOMOROWSKI, S. F. COOKE, E. KAPLAN, M. F. BEAR;
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Abstract: Visual experience in the mouse can induce long-term changes in the responsiveness of primary visual cortex (V1) to a specific visual stimulus through a process called stimulus-selective response potentiation (SRP). This plasticity is thought to reflect the neural substrate of a simple visual memory stored within V1 that plays a fundamental role in the mouse's ability to behaviorally habituate to a familiar visual stimulus. Recent work in mouse V1 has revealed that interactions between the oscillatory state of cortex and an animal's behavior can have a profound impact on the processing of visual information. Here we examine the relationship between oscillatory state and neural activity in visual cortex in the context of SRP through spiking and local field potential (LFP) activity within mouse V1. We find that LFP recorded from thalamo-recipient layer IV of V1 alternates between two major states, one dominated by high-amplitude, low-frequency rhythms between 6 and 10Hz known as High-Voltage Slow oscillations (HVS) and one dominated by high-frequency (60-100Hz) gamma rhythms (HG), which reflect very different underlying spiking activity: HVS are associated largely with bursting activity in V1 and HG states are associated with tonic firing. These two states reflect very different processing modes within V1. The oscillatory state of V1 dramatically shifts towards an HVS-dominated state as a stimulus becomes progressively familiar. However, the presentation of novelty, even simply through a shift in stimulus orientation, can very swiftly shift the cortex back into a gamma-dominated state. Importantly, we show that SRP is only observable in the HG state and not the HVS state. These shifts in oscillatory state also correlate strongly with mouse behavior elicited by the onset of the visual stimulus. Our results indicate that oscillations in V1 reflect dynamics in stimulus coding within V1 that are highly modifiable by experience. Furthermore, these changes may be adaptive, allowing the animal to identify and encode information about novel and salient stimuli while conversely shifting into an offline state when information is highly familiar.

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Poster

641. Learning and Memory: Sensory and Association Cortex

Location: Halls B-H

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Program#/Poster#: 641.11/KKK5

Topic: H.01. Animal Cognition and Behavior

Title: Modeling perceptual learning in V4 based on changes in causal relationships between neurons

Authors: *J. D. YOUNG[1], V. DRAGOI[2], B. AAZHANG[1];
[1]ECE, Rice Univ., Houston, TX; [2]Neurobio. and Anat., Univ. of Texas-Houston Med. Sch., Houston, TX

Abstract: Perceptual learning is a well observed phenomenon but is poorly understood in terms of neurophysiology. We seek to create a model of perceptual learning in V4 based on neuronal influence. Previous research seeking to discover a neuronal explanation for visual task learning, a subset of perceptual learning, involved rhesus macaques repeatedly attempting a visual task. Monkeys were shown an image on-screen, then soon after shown a potentially rotated version of the same image and given the opportunity to identify the presence of a rotation. Analysis of local field potentials and spike timings revealed heightened local synchronization between V4 neurons during learning. We use directed information (DI), an information theoretic tool, on the same data set to quantify how the influence structure between V4 neurons changes over the time course of perceptual learning. DI is ideal for this application due its ability to compute the causal effect of one time series on another with minimal assumptions and its capacity to condition on other time series. Estimation of DI is based on a previously published technique that utilizes context-tree weighting in order to estimate probabilities for entropy estimation and therefore DI estimation.
By estimating the DI rate between neuron spike trains, we reveal different periods of DI rate change for select neuron pairs. Early and late trials of a given experiment show DI rate variation while middle trials exhibit stable DI rates. Overall, these neuron pairs exhibit significant feedback relationships. Results are compared with the accuracy of the monkey's decisions in order to determine correlation between DI rate trends and perceptual learning. We then develop a temporal network model of the time course of perceptual learning in V4. We use directed edges with weights proportional to the aforementioned changes in neuronal influence quantified by DI rate. We will test our model in rhesus macaques by stimulating V4 neurons to create the newly observed influence structure while they perform a visual task. Our stimulation technique will result in a faster perceptual learning rate. Furthermore, stimulation will restore learning capability in cases where individuals suffer from learning disabilities.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: CIHR

Title: An immunohistochemical approach for quantifying excitatory and inhibitory neurotransmission in areas MT and MST of the macaque monkey

Authors: *N. MALEK[1], J. NOVEK[2], R. MCKINNEY[3], M. PETRIDES[2], J. MARTINEZ-TRUJILLO[4];
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Abstract: Functional characterizations of lower-order visual area MT (middle temporal) and higher-order multimodal association area MST (medial superior temporal) have been based on electrophysiological recordings, including mapping of receptive field size and motion-direction tuning. However, less is known about the comparative anatomy of both these areas. A recent study suggests differences in the functional roles of areas MT and MST in the macaque monkey for the coding of working memory (Mendoza-Halliday et al., Nat. Neuro., 2014), possibly due to differences in the proportion of excitatory and inhibitory neurotransmission. Previous quantitative histological examinations of excitation and inhibition have used few sampling locations at very high magnification to extrapolate characteristics of entire areas. Furthermore, it is often not known in which cortical layers such images are sampled. In order to gain a better understanding of excitation and inhibition differences in areas MT and MST in rhesus macaque monkeys, we pursued a more global approach that combined immunohistochemical staining and widefield imaging. To visualize local inhibitory expression, we targeted the main inhibitory neurotransmitter GABA with the immunohistochemical stain VGAT. To visualize excitation expression, for which glutamate is the primary neurotransmitter, we used VGlut1 and VGlut2 to stain for intracortical and thalamic inputs, respectively. Widefield fluorescence images of the entire areas of interest were acquired, cortical layers were manually drawn in, and evenly spaced sampling lines were used to acquire pixel intensity values across the cortical depth for all three fluorescence channels. Normalized profiles were obtained for each area and then statistically analyzed. We found differences in relative excitation and inhibition between MT and MST with the VGAT and VGlut1 stains, with the most apparent differences being found in layers II and III. These results provide anatomical evidence supporting the functional differences observed between these two areas.

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Poster

641. Learning and Memory: Sensory and Association Cortex

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NINDS NS059312

McKnight Foundation

Title: Working memory related neuronal activity is widely distributed throughout cortex during an object-based delayed match-to-sample task in the macaque monkey

Authors: *C. M. GRAY, N. DOTSON, S. HOFFMAN;
Cell Biol. and Neurosci., Montana State Univ. Bozeman, Bozeman, MT

Abstract: Task and stimulus dependent neuronal activity that continues during the memory period of a delayed match-to-sample task is considered to be the quintessential neural correlate of working memory. This persistent, mnemonic activity has been most extensively studied in prefrontal and posterior parietal cortical areas, but less so in other regions of the cortex. Consequently, much remains to be learned regarding the distribution of persistent activity. Recently, we developed a large-scale semi-chronic recording system that enables the long-term measurement of neuronal activity from up to 256 independently movable microelectrodes spanning an entire cerebral hemisphere in non-human primates. We implanted this instrument in two macaque monkeys and recorded neuronal activity for 6 and 10 months, respectively, while the animals performed an object-based, delayed match-to-sample (dMTS) task and a set of control tasks.

At the end of the experiments, the electrode tracks were identified histologically and the cortical area of each recording site was reconstructed using the Caret software package. Based on these analyses, we obtained recordings of neuronal activity from 63 separate cortical areas and 4 subcortical structures include the Caudate nucleus, Putamen, Claustrum and Thalamus. The cortical areas varied in location from V1 to area 11 along the AP axis, TEOm to area 23 along the ML axis, and F1 to TEpd along the VM axis.

We extracted single- and multi-unit activity from each recording site and determined the incidence of task-dependent and stimulus-specific activity during all phases of the task using a sliding window analysis of firing rate (Kruskal-Wallis test ($P<.05$) with correction for the false discovery rate).

These analyses revealed a widespread, but sparse distribution of task dependent and stimulus specific changes in neuronal activity throughout the cortex. Sample specific activity was most

widespread, including areas throughout primary and extrastriate visual cortex, posterior parietal areas, motor and premotor areas, prefrontal cortices and Basal Ganglia. Task and sample specific delay period activity occurred less often, but with a similar distribution, and was composed of both increases and decreases in firing rate. Sample specific delay period activity occurred throughout multiple areas of visual cortex, including striate cortex, and a number of premotor and prefrontal areas not previously described for this task.
These findings demonstrate that visual working memory-related activity is widespread throughout many regions of the cortex suggesting that short-term mnemonic representations are distributed in nature.

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Poster

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Topic: H.01. Animal Cognition and Behavior

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Title: Role of insular cortex in emotional learning

Authors: *Y. CHEN[1,2], C. S. PÉREZ[4], F. MALTESE[2], N. NADIF KASRI[2,3], B. ROOZENDAAL[2];
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Abstract: Extensive evidence from neuroimaging studies in humans consistently point toward a key role for the insular cortex (IC) in the detection of novel and salient information. Yet, little is known whether the IC is also involved in emotionally influenced learning and memory. The present study investigated whether noradrenergic activation, which is normally induced during an emotionally arousing situation, in the IC is required for the consolidation of object recognition memory. Male Sprague-Dawley rats were trained on an object recognition task for either 3 or 10 min during which they could explore two identical objects. Immediately after the training they received bilateral intra-IC infusions of norepinephrine (1.0 μg) or the β-adrenoceptor antagonist propranolol (0.3 μg). Saline-infused control rats exhibited poor 24-h retention when given 3 min of training and good retention when given 10 min of training. Norepinephrine administered after 3 min of object training enhanced retention, whereas propranolol administered after 10 min of

training produced memory impairment. Moreover, we found that in untreated groups of trained rats 10 min of object recognition training increased neuronal activity in the IC 1 h later, assessed by the number of cells expressing immunoreactivity for phosphorylated cyclic-AMP response element-binding protein (pCREB).These findings indicate that the IC is involved in regulating emotional arousal effects on the consolidation of object recognition memory.

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Poster

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Title: A whisker-mediated spatial localization task for investigating how mice associate tactile stimuli with their behavioral relevance

Authors: ***G. M. PIERCE**[1,2], A. K. KINNISCHTZKE[1,2], R. M. BRUNO[1,2];
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Abstract: Mice use their whiskers to explore their surroundings and to identify the objects around them. As mice move through the environment, they must be able to attend toward and selectively respond to the small fraction of objects that are relevant to their goals. To investigate how the somatosensory system associates objects with their behavioral relevance (i.e. whether objects predict reward outcomes), we developed a head-fixed, whisker-based behavioral paradigm in which mice respond to a relevant target object and ignore a distractor.
Head-fixed behavioral tasks are useful for studying whisker-mediated behaviors because they allow for precise presentation of stimuli and permit a diverse array of techniques for recording and manipulating neural activity. Most common head-fixed behavioral paradigms require mice to discriminate between two objects by responding to one and suppressing a response to another. In such tasks, both objects are equally informative about the correct response. In contrast, we have developed a spatial localization task in which only one object (the “target”) is informative about the correct response, while a simultaneously presented “distractor” object is irrelevant. These

two objects are identical in physical characteristics (shape, size, texture, etc.) and differ only in their relevance to the animal. Mice distinguish between the two identical stimuli based on their location in the whisker field and are able to learn the association between the presence of the target and the possibility of reward. Furthermore, mice do not base their behavior on the presence of the distractor, but rather react only to the presence of the target. This task will facilitate investigation of how somatosensory thalamus, cortex, and other brain structures encode relevant tactile objects and behavioral choice. Such experiments will shed light on how the brain associates objects with their behavioral relevance, allowing animals to respond selectively to important stimuli in their environment.

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Poster

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Title: Green light treatment improves learning and memory in mice

Authors: M. TAN[1], K. CHEN[1], L. HUANG[1], L. ZHANG[1], A. LI[1], K.-F. SO[1], H. GU[2], *C. REN[1];
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Abstract: Purpose: Phototherapy has been shown to be effective for a variety of mental diseases. For example, blue light treatment can effectively improve learning and memory functions in Alzheimer's patients. However, the known retinal damage effect of blue light

stimulation limits its application. In this study, we thus investigated the effect of green light (527nm) illumination on learning and memory functions in mice. **Method:**Thy-1 H line mice were randomly divided into three groups, which received room light, blue light and green light illumination using LED sources (2.2 μmol/ m^2·s in photon density) for two consecutive weeks (12 h light / 12 h dark normal cycle). The ability of learning and memory was assessed using whisker-dependent novel texture discrimination task. Moreover, the dendritic spine plasticity of layer V pyramidal neurons in barrel cortex was examined *in vivo* using two-photon microscopy to analyze formation, elimination and turnover rate of spines. **Result:** Whisker-dependent novel texture discrimination task showed that green-light treatment significantly shortened the time for learning novel texture compared to that in room-light treatment animals. Moreover, green-light illumination also extended the retention time of novel texture memory compared to room-light treatment group. Two-photon microscopy revealed similar spine formation rates among all groups ($p>0.05$). There was significantly higher elimination rate in green light or blue light treatment mice compared to room light animals ($p<0.05$). Spine turnover rate was similar between green and blue light groups, both of which were higher than room light group ($p<0.05$). No significant difference of spine dynamic parameters has been found between green and blue light group ($p>0.05$). **Conclusion:** Green light illumination could improve memory and learning abilities of novel texture in mice, possibly via increasing spine turnover rate.

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Poster

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Title: Conditional knock-out of the ACAN gene removes aggrecan and WFA-positive perineuronal nets in adult mice

Authors: ***M. H. FYHN**[1], K. LENSJØ[1], D. ROWLANDS[2], V. DINH[3], M. R. ANDREWS[4], T. HAFTING FYHN[3], J. W. FAWCETT[2], G. DICK[1];
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Abstract: Perineuronal nets (PNNs) are a specialized form of extracellular matrix in the CNS that mainly enwraps parvalbumin (PV) expressing inhibitory interneurons. They assemble in parallel with the maturation of the inhibitory network and with the closure of critical period plasticity. Several lines of evidence support a role for PNNs in stabilizing synapses, limiting adult brain plasticity and in some neurological disorders. While the mature PNN is a complex structure of several components, recent work suggests that the proteoglycan aggrecan, a product of the ACAN gene, is vital for the PNNs. However, because aggrecan is essential to cartilage throughout the body, global ACAN knock-out is lethal. Thus, to investigate the role of aggrecan for PNN assembly and stability, and its contribution to brain plasticity, we have established a CRE-inducible conditional ACAN knock-out mouse. The transgenic construct is a design of the European Conditional Mouse Mutagenesis Program and has had two loxP sites inserted into the genome, flanking exon 4 of the ACAN gene. Two approaches were used to remove aggrecan from the conditional ACAN knock-out mouse, either by (1) injecting a viral vector expressing CRE recombinase under control of the synapsin promoter to obtain area specific knock-outs in adult mice, or by (2) crossing with the PV-Cre line, which expresses CRE recombinase downstream of the PV transcript. Aggrecan and PNNs was determined by immunohistochemical staining against aggrecan, PV and the PNN specific lectin *Wisteria floribunda* agglutinin. Conditional knock-out of ACAN efficiently eliminated both aggrecan and PNNs with both approaches, while the PV staining intensity remained unaltered. Adult knock-out ACAN/PV-Cre mice were lacking PNNs. This shows for the first time that aggrecan is produced by the PV neurons themselves, in line with a role of PV neurons in regulating brain plasticity. To investigate the role of aggrecan for plasticity we restricted the knock-out of the ACAN gene to the visual cortex of adult mice by using local injections of viral vectors with Cre-recombinase. Activity-dependent plasticity was induced by monocular deprivation and assessed by intrinsic optical signal imaging. Preliminary results indicate that knocking out ACAN in adult visual cortex reinstate a high level of plasticity similar to that of juveniles. Taken together, the conditional ACAN knock-out mouse can be used to target the ACAN gene, leading to loss of aggrecan and abolished PNNs as well as increased plasticity. Our results indicate that aggrecan is vital for the assembly and stability of PNNs, making the ACAN mouse a robust tool to reveal the role of PNNs in plasticity and disease.

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Poster

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Title: The perineuronal nets in the lateral secondary visual cortex are essential for remote visual fear memory

Authors: *K. K. LENSJØ, M. WIGESTRAND, E. THOMPSON, A. MALTHE-SØRENSEN, T. HAFTING, M. FYHN;
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Abstract: Compared to our knowledge of the molecular mechanisms underlying learning and memory encoding and consolidation, far less is known about remote memory storage. Recently, it was proposed that the specialized extracellular matrix structures called perineuronal nets (PNNs) may be a physical framework for long term memory storage (Tsien, 2013). Their mesh like structure, tightly enwrapping and stabilizing synaptic connections and their composition of molecules with very slow turnover rates, point in this direction but the idea remains to be tested. Here, we test this hypothesis by investigating remote visual fear memories. Recent evidence indicate that over time, visual fear memories become dependent on the lateral part of the secondary visual cortex (V2L), suggesting that secondary sensory cortices may be pivotal in long term memory storage. In order to investigate the contribution of PNNs for memory stability, we induced a remote visual fear memory in adult Sprague Dawley rats using Pavlovian fear conditioning and experimentally degraded the PNNs by local injections of the enzyme Chondroitinase ABC (chABC) into V2L. Injections of chABC were performed at different time-points in the fear conditioning learning paradigm; either prior to training or prior to retrieval of the remote memory. We found that degrading the PNNs in V2L prior to the remote memory retrieval test selectively disrupted the visual fear memory while degrading the PNNs prior to training had no effect on memory retrieval. Moreover, degrading the PNNs in the primary visual cortex had no effect on the remote memory. In order to elucidate the neural network responses underlying the impaired recall we use chronically implanted electrodes and perform simultaneous local field potential recordings from V2L and the basolateral amygdala. Together, these findings indicate that the PNNs in V2L are critical for visual fear memory processing at remote time-points, but not for memory acquisition or early consolidation.

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Poster

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Support: National Institute of Health (NIDCD) 1 R03 DC014753-01

Title: Epigenetic mechanisms gate auditory cue-reward extinction and sensory cortical plasticity

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Abstract: Cortical plasticity is dynamic, changing the representation of memory based on a lifetime history of experiences. Behaviorally-relevant sensory cues are transient, but can be transformed into long-term memory (LTM). Formation of LTM requires neuroplasticity that changes neural function, structure and connectivity. At a molecular level, long-term changes require gene expression. Previous research has shown that epigenetic mechanisms can selectively regulate gene expression. For example, a class I histone deacetylase (HDAC) called HDAC3 seems to be a "molecular brake" on plasticity required for LTM (McQuown et al. 2011). Blocking HDAC3 with the drug RGFP966 releases the brakes on gene expression enabling highly cue-specific LTM and cortical reorganization in the primary auditory cortex (A1) for the sound frequency of a salient cue. More cells in A1 tonotopy can become "re-tuned" to the frequency of a cue signaling a reward (Bieszczad & Weinberger 2010). Yet, tonotopy can also reorganize in the opposite direction to reduce the number of cells "tuned" to the frequency of a cue after it is extinguished. There is a significant positive relationship between the magnitude of frequency representational area in A1 and that frequency's strength in associative memory, whether in cue-reward acquisition or in cue extinction. Previous work has shown that HDAC3 inhibition via RGFP966 can enable cue-specific cortical plasticity with cue-reward acquisition learning (Bieszczad et al. 2015). The purpose of this study is to test whether HDAC3 has similar control over the cue-specific cortical plasticity for extinction, which is predicted to strengthen extinction memory. To test this hypothesis, male Sprague Dawley rats are trained to bar-press for a water reward in response to a 5.0 kHz (70 dB) pure tone in a first phase of training, and are later trained in a second phase to extinguish the bar-press response learned by the cue-reward association. During extinction training, one group of animals is injected with RGFP966 (10mg/kg; i.p.) and the other group with vehicle. A spontaneous recovery test is used to determine the strength of cue-specific extinction. A1 electrophysiology after extinction training and memory testing determine frequency-specific tonotopic re-organization. Data presented will show whether HDAC3 regulates both extinction and acquisition memory. These results are relevant in work towards a comprehensive model of the function and epigenetic regulation of

sensory cortices and their role in cued memory. It also has applications in development of treatments for cue-related addictive behaviors, and cued emotional stressors in PTSD.

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Poster

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Title: HDAC3 dynamically regulates discrimination learning and information storage in primary auditory cortex

Authors: *A. SHANG, K. M. BIESZCZAD;
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Abstract: Epigenetic mechanisms that modulate gene expression – such as histone modification – are key for regulating the neuroplasticity underlying long-term memory (LTM) formation. Blocking histone deacetylases (HDACs) has been shown to facilitate various forms of LTM by *releasing the brakes* on neuroplasticity (e.g. McQuown et al., 2011; Stefanko et al., 2009). Recent work in the auditory system using a simple single-tone associative learning paradigm suggests that pharmacological inhibition of HDAC3 via RGFP966 can enhance the specificity of memory for relevant sound features, and also reorganize primary auditory cortex (A1) to enhance the representation of those features (Bieszczad et al., 2015).
We designed a two-tone frequency-discrimination (2TD) task to determine if HDAC3 regulates auditory discrimination learning. This instrumental learning task can also determine the temporal dynamics of HDAC3 action on 2TD acquisition, performance and underlying plasticity in A1. Rats (male; Sprague-Dawley) learn to discriminate between two spectrally-distant frequencies: bar-press (BP) responses to only the CS+ (5 kHz) are rewarded; BPs to the second sound, CS- (11.5 kHz), result in a time-out (extended time until next trial). Sessions are followed by injections of RGFP966 (10mg/kg; *i.p.*) or vehicle (Veh). Frequency-specificity of the auditory memory formed is assessed after two weeks of daily training sessions using a stimulus generalization (SG) test, where a range of test frequencies (including the CS+ and CS-) are presented under extinction conditions. Electrophysiological recordings show the tonotopic

effects in A1 that could underlie differences in behavior.
Differences in behavioral (2TD acquisition & performance; SG) and neural (A1 tonotopic reorganization) frequency-specificity between groups will support the hypothesis that HDAC3 is a molecular epigenetic regulator of sensory information consolidation during incremental learning experiences. Data presented will show whether RGFP966-treated rats have rapid 2TD acquisition and reach asymptotic levels of performance sooner than Veh-treated rats. SG may likewise reveal sharper (more frequency-specific) memory, with more selective BPs to the CS+. Unusually rapid and frequency-specific reorganizations in A1 for the salient sounds are expected to underlie frequency-specific behavioral effects of HDAC3. Such results further specify a role for epigenetic mechanisms in learning, memory, and information storage. Furthermore, they initiate a novel investigation into the temporal dynamics of epigenetic regulators that convert the sensory details of transient experiences into LTM.

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Title: Histone modification enables song-specific auditory memories in an avian model.

Authors: ***M. L. PHAN**, J. JIMÉNEZ CASTILLO, S. MAHIDADIA, S. SAAD, D. S. VICARIO, K. M. BIESZCZAD;
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Abstract: Vocal communication relies on the brain's ability to process, learn and remember important sounds. Long-term memories (LTM) of salient sounds require the expression of genes that subserve stable changes in neural function, structure, and connectivity in the auditory areas of the brain. Molecular epigenetic mechanisms regulate gene expression required for this memory formation. For example, the enzyme histone deacetylase 3 (HDAC3) gates the transformation of an auditory experience into memory. Bieszczad et al. (2015) demonstrated that rats treated with a selective HDAC3-inhibitor (RGFP966) acquired a specific and unusually

detailed auditory associative memory for pure tones, which occurred with highly specific neuroplasticity in primary auditory cortex (A1).
We test the hypothesis that HDAC3-inhibition enables long-term neuronal memories for ethologically-relevant communication signals with complex acoustic features (Phan & Bieszczad 2016), using an avian model. The songbird caudomedial nidopallium (NCM) is analogous to superficial layers of mammalian A1 or to a secondary auditory cortex and contributes to auditory discrimination and song memory. Neurons in NCM undergo neuroplasticity in the form of stimulus-specific adaptation (SSA). SSA occurs when presented with repeated song stimuli, evoked responses to familiar (F) stimuli adapt more slowly than to novel (N) stimuli. Comparison of adaptation rate (the slope of the decrease in response amplitude as a function of repetition number) between F and N songs provides a measure of the strength of song memories. Normally, 200 repetitions of a conspecific song will lead to LTM measured ~20h later. We exposed adult male zebra finches (n=7) to ≤ 40 repetitions of 8 novel zebra finch songs, below the usual threshold for memory formation. Systemic injection of RGFP966 (n=3) or vehicle control (n=4) followed exposure. Neural responses in NCM were recorded 20h later to the 8 previously exposed F songs and to 8 never-before-heard N songs. In vehicle treated birds, SSA rates were not significantly different between the N and F songs, indicating no memory of the F stimuli (K-S test; p=0.575, D=0.084, Z=0.740). In birds injected with RGFP966, adaptation rates were shallower (less negative) for F stimuli than for N stimuli (K-S test; p=0.016, D=0.206, Z=1.50). This difference indicates that RGFP966 treatment enabled memories for specific song stimuli. Therefore, HDAC3-inhibition can effectively transform a sub-threshold auditory experience (minimal repetitions of song exposure) into a LTM of that experience, apparently by lowering the threshold number of exposure events required for memory to form.

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Poster

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Title: Functional footprints of impaired consciousness in mice with reduced molecular diversity of clustered protocadherin-α

Authors: *K. SHIBUKI[1,2], T. YAMAGISHI[3], D. KAMATANI[1,2], K. YOSHITAKE[1,2], H. TSUKANO[1], K. WATANABE[1], R. HISHIDA[1], K. TAKAHASHI[3], S. TAKAHASHI[3], A. HORII[3], T. YAGI[4,2];
[1]Brain Res. Inst., Niigata, Japan; [2]CREST/AMED, Tokyo, Japan; [3]Niigata Univ., Niigata, Japan; [4]Osaka Univ., Osaka, Japan

Abstract: The integrated information theory of consciousness suggests that various types of dynamic short-term memory highly integrated together in the brain produce consciousness. However, the presence or absence of specific neural mechanisms of consciousness is unknown. If complex neural circuits conceive consciousness in principle, consciousness is tightly bound to other neural functions, as observed during anesthesia or sleep. If consciousness requires specific neural mechanisms, it may be separated from other neural functions, i.e. some genetically manipulated mice with apparently normal neural functions may have impaired consciousness. We report evidences supporting the latter possibility. Clustered protocadherins (cPcdhs, α-γ) are neuro-specific cell adhesion molecules characterized by clustered gene structures. In each neuron, some of the gene clusters are randomly selected and determine the specificity of cell adhesion properties. These molecules have been supposed to play a role in formation of sophisticated neural circuits. In cPcdhα1,12 mice, only cPcdhα1 and cPcdhα12 (but not cPcdhα2- cPcdhα11) are expressed, while the total amount of cPcdhα is constant. These mice exhibit no apparent abnormality at glance. However, we found that visual short-term memory was clearly impaired in behavioral experiments using spatial cues or shape cues. Visual short-term memory of spatial information was evaluated using a T-maze test. Although cPcdhα1,12 mice exhibited a comparable performance with that of control mice in a visually-guided task, their performance was significantly impaired in a memory-guided task. Visual short-term memory of shape information was evaluated using a delayed match-to-sample test, and cPcdhα1,12 mice exhibited a nice performance in a control task with no delay while the performance was significantly impaired in a task with a delay for 20 s. Furthermore, cortical plasticity and behaviors depending on multimodal sensory integration between visual and whisker information or between visual and auditory information were clearly impaired. Since cPcdhα is distributed all over the brain, various types of short-term memory and sensory integration are likely impaired in cPcdhα1,12 mice. These results strongly suggest that specific neural mechanisms depending on molecular diversity of cPcdhα are required for producing consciousness. The present study discloses functional footprints of impaired consciousness in mice, and we hope that these footprints could be a useful key for elucidating the neural mechanisms of consciousness at cellular and molecular levels.

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Poster

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Title: High-throughput phenotypic profiling of genes implicated in Autism Spectrum Disorders identifies genes important for habituation

Authors: *T. MCDIARMID[1], C. H. RANKIN[2];
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Abstract: A major challenge to finding the causes of Autism Spectrum Disorders (ASD) is that thousands of gene mutations have been linked to the disease. To find causal links between mutations and symptoms we need a system in which we can rapidly manipulate genetic variation and measure the resulting phenotype. One endophenotype found in ASD is decreased habituation. Habituation is a form of learning observed as a decrease in responding to repeated stimulation. Habituation has been conserved through evolution, making it amenable to study using genetic model organisms such as *Caenorhabditis elegans*. Here, we use a machine vision system, the multi-worm tracker, to assay mechanosensory habituation in 52 strains of *C. elegans* each carrying a mutation in an ortholog of an ASD-linked gene to determine which genes disrupt habituation when mutated. Our screen identified several habituation mutants from the ASD-linked genes; these genes fall into three main functional groups: cell-adhesion molecules, ion channels, and transcription factors. Of note, CTNNB1(*bar-1*), FAT3(*cdh-4*), and CREBBP(*cbp-1*) mutants all showed decreased habituation across multiple behavioural measures. Rescuing *C. elegans* disrupted habituation phenotypes with orthologous human genes will confirm functional homology between *C. elegans* and human genes. If the human gene rescues the worm phenotype we can then rescue with human variants found in ASD and categorize them in to variants that rescue and those that do not. This will allow us to group variants into functional categories that may shed light on their role in ASD.

Disclosures: T. McDiarmid: None. **C.H. Rankin:** None.

Poster

642. Learning and Memory: Invertebrates

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

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Topic: H.01. Animal Cognition and Behavior

Support: NSERC Discovery Grant #122216-2013

Title: Alcohol's effect on habituation involve Ga/cAMP/PLA and the Gao/DGA/DAG pathway in *Caenorhabditis elegans*

Authors: C. LIN[1], S. MIRHADI[2], S. SOO[2], *C. H. RANKIN[1];
[1]Univ. British Columbia, Vancouver, BC, Canada; [2]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Alcohol intoxication impairs learning, but the underlying mechanism is not well understood. Habituation is a type of learning measured as a gradual decrease in response to repeated stimuli that is not due to fatigue or adaptation. Using the genetic model organism *Caenorhabditis elegans*, we investigated the roles of several candidate genes in the effect of alcohol on habituation. Animals were exposed to 400mM ethanol for 30mins (~0.08% BAC) and then their behavior and responses to 30 mechanical stimuli (taps) delivered at 10s Inter-stimulus interval were tracked using the Multi-Worm Tracker. Wildtype animals respond to the tap stimuli by moving backwards (reversals), and the probability and magnitude of this reversal response gradually decrease as the number of tap increases. On alcohol, wildtype worms habituate to the taps faster and more deeply than unexposed animals. Interestingly, wildtype worms on alcohol also shifted their responses to taps from reversals to faster forward movement over the course of habituation. Our initial genetic analysis showed that some genes implicated in other behavioral effects of alcohol in C. elegans were also involved in alcohol-dependent habituation phenotypes (BK channel, Neuropeptide Y receptor), however some other implicated genes were not (D1 receptor. We tested the role of the Gαs/cAMP/PKA pathway and Gαo/DGK/DAG pathway in alcohols effects on habituation. In the Gαs/cAMP/PKA pathway, Gαs (*gsa-1),* adenylyl cyclase (*acy-1)* and the negative regulator cAMP phosphodiesterase (*pde-4),* were both found to be involved in the deeper habituation induced by alcohol, however the regulatory subunit of PKA (*kin-2)* may not to be. In the Gαo/DGK/DAG pathway, Gαo *(goa-1)* was found to be involved in the deeper habituation induced by alcohol, but another member of the pathway, *unc-13,* was not. This study identified novel mechanisms underlying learning impairment under alcohol intoxication.

Disclosures: C. Lin: None. **S. Mirhadi:** None. **S. Soo:** None. **C.H. Rankin:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: NSERC Discovery Grant #122216-2013

Title: A neuropeptide mediated integration of habituation and sensitization to repeated optogenetic stimulation promotes escape in *C. elegans*

Authors: *A. YU, E. ARDIEL, C. RANKIN;
The Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Habituation is a highly conserved, but poorly understood phenomenon. To identify molecular components mediating habituation, we took advantage of a high-throughput *C. elegans* learning assay using optogenetics for controlled neuronal activation and real-time computer vision software for automated behavioral tracking. We implicated neuropeptides in habituation of ASH-mediated reversals and performed a targeted RNAi screen to identify candidate receptors. Through subsequent mutant analysis and cell-type specific rescue, we show that pigment dispersing factor (PDF) ligands function redundantly to promote habituation via PDFR-1-mediated cAMP signaling in both neurons and muscles. Behavioral analyses of a number of components of the locomotion during learning acquisition indicated that different components of the behavior showed different patterns of plasticity. Mutant analyses and rescues revealed that aspects of response habituation occur primarily in neurons and locomotion sensitization primarily in muscle as part of a shifting behavioral strategy orchestrated by PDF signaling to promote dispersal. This suggests that habituation reflects a coordinated change in several behaviors to produce a coordinated response. This is in contrast to the traditional definition of habituation as learning to ignore stimuli. In addition, it leads to questioning single response-centric analyses of learning.

Disclosures: **A. Yu:** None. **E. Ardiel:** None. **C. Rankin:** None.

Poster

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Support: NSF

NIH

Title: Molecular organization of Octopus brains reveals first insight into unique memory center signaling

Authors: *G. C. WINTERS[1,3], A. B. KOHN[2], G. POLESE[4], A. DICOSMO[5], B. HOCHNER[6], N. STERN[6], R. LAUX[7], L. L. MOROZ[1,3];
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Abstract: Cephalopods (*Octopus*, Squid, Cuttlefish, and *Nautilus*) have evolved a degree of behavioral flexibility that rivals that of many mammals. The Vertical Lobe (VL), a structure unique to cephalopods containing intricate memory circuitry, parallels mammalian analogues like the hippocampus in cell number and function, but has evolved independently within the distant molluscan lineage. We used integrative Next-gen sequencing technology and bioinformatic analyses, followed by anatomical validation using in-situ hybridization, to identify the first molecular maps of signaling molecules implemented in cephalopod memory circuitry. We constructed, sequenced and analyzed *Octopus* neural transcriptomes of various tissues (including VL, CNS, SFL-Superior Frontal lobe, and Arm Cords), and individual cells from sub-populations within the VL and other neuronal tissues, including the Amacrine (Am) Interneurons and Large Efferent (LE) Neurons that make up the VL. We compared these transcriptomes to the publically available *Octopus* genome and our gastropod mollusc neural transcriptomes including *Aplysia californica*. We identified 16,194 transcripts in the VL (RPKM >/=1) and found 4,139 (25.5%) appear to be cephalopod-specific. Remarkably, indicators for many memory related signal molecules and transmitters like NO (NOS), GABA (GAD), and acetylcholine (ChAT) were not identified in VL transcriptomes, suggesting a distinct cephalopod-specific complement of molecules independently recruited in the organization of learning and memory-forming circuits. We used the well characterized neuropeptide (NP) precursor complement of *Aplysia* to find 22 homologs in *Octopus* VL and 23 in the SFL. We have systematically cloned and mapped expression of NPs, and have localized 9 NP to the components of the VL circuit. NPX3 is abundantly expressed in the cell bodies of the MSF, where the afferent tract the VL originates

and NPX1 localizes to the cell bodies of each VL gyrus. Next we used unbiased computational predictions and manual annotation to identify putative signaling molecules form the VL transcriptomes. From this we have identified 4 cephalopod-specific abundant transcripts, one of which, NPX2 localizes to all cell bodies in the VL and Subfrontal lobes. Among the 6 NPs that localize to the VL LE neuron, 2 unique cephalopod NPs, NPX4 and 5, are abundant in *Octopus*, but absent in the ancestrally branching Nautilus CNS (which lacks a VL). This expansion of novel signaling molecules in the VL circuit is likely a key feature of the unique memory systems of cephalopods, further implying extensive parallel evolution of cephalopod brains and memory circuits in particular.

Disclosures: G.C. Winters: None. **A.B. Kohn:** None. **G. Polese:** None. **A. DiCosmo:** None. **B. Hochner:** None. **N. Stern:** None. **R. Laux:** None. **L.L. Moroz:** None.

Poster

642. Learning and Memory: Invertebrates

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Program#/Poster#: 642.05/KKK21

Topic: H.01. Animal Cognition and Behavior

Title: Pavlovian fear conditioning in the terrestrial caribbean hermit crab coenobita clypeatus

Authors: *A. P. BLAISDELL, M. T. DO, L. J. TEOPENGCO, J. E. SCHROEDER; Psychology, UCLA, Los Angeles, CA

Abstract: *Coenobita clypeatus* is a species of terrestrial Caribbean hermit crab previously shown in our lab to emit defensive behavior to a threatening visual stimulus. We investigated fear conditioning in *C. clypeatus* using an audiovisual cue as the conditioned stimulus (CS) and being agitated in a small cup as the unconditioned stimulus (US). In the experimental Group Paired, the CS was paired with the US. In the control Group Unpaired, the CS and US were presented on separate trials in an explicitly unpaired manner. Conditioning consisted of one session of 10 trials. For Group Paired, each trial consisted of placing the subject in a clear plastic cup attached to a rod. After the crab emerged from its shell, the CS was presented for 5 s. 2-s after the onset of the CS, the cup was agitated by gently manually rotating the rod for 3 s. At the end of the trial, the CS and US co-terminated. In Group Unpaired, each trial consisted either of a 5-s presentation of the CS or a 3-s presentation of the US, with five of each type of trial, their order pseudorandomized in the session. For both groups, a 60-s intertrial interval (ITI) separated each trial. Presentation of the agitation US caused crabs to hide in their shell (the unconditioned response). For all subjects in Group Paired, but none in Group Unpaired, hiding or freezing responses to the CS prior to the onset of the US developed over the course of 10 trials. We report

on the features of the CS that control the conditioned response, and the duration of learning. This procedure produces strong and reliable fear conditioning in *C. clypeatus*, and will allow for investigations into the psychological and neural basis of Pavlovian conditioning in the terrestrial hermit crab.

Disclosures: A.P. Blaisdell: None. **M.T. Do:** None. **L.J. Teopengco:** None. **J.E. Schroeder:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: RFBR Grant 16-04-01517

Title: MK-801 and methiothepin immediately before reconsolidation produce different types of amnesia in snail Helix lucorum

Authors: *S. SOLNTSEVA[1,2], A. V. SHEVELKIN[1,3], O. I. EFIMOVA[4], P. V. NIKITIN[1], S. A. KOZYREV[1], V. P. NIKITIN[1];
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Abstract: Considerable progress has been made in understanding the neurophysiological and molecular mechanisms of long-term memory formation and stabilization. Amnestic processes such as memory loss, forgetting, and amnesia, are far less extensively studied, despite their theoretical, social, and medical importance.
We studied time course of amnesia produced by impairment of memory reconsolidation by NMDA glutamate receptor antagonist (MK-801) or 5-HT/dopamine receptor antagonist (methiothepin) immediately before reminder in snails *Helix lucorum*. Amnesia was induced by MK-801 or methiothepin with following reminder procedure two days after food aversion conditioning. We attempted to train these snails again three or ten days after amnesia induction and found faster learning than at the initial training at 3rd day after amnesia induction. However, 10 days after the methiothepin/reminder, we observed that time course of food aversion conditioning was similar to that at initial training. While, the repeated training 10 days after MK-801/reminder did not produce long-term memory in snail at all.
Thus, we demonstrated that impairment of memory reconsolidation by 5-HT/dopamine receptor

antagonist/reminder or NMDA glutamate receptors antagonist/reminder induces different types of amnesia. Both types of amnesia have similar time course of repeated learning at early period of time (it's successful and proceed faster), but they are different at late period of time (learning is successful only after methiothepin/reminder but not after MK-801/reminder). Our results suggest that the treatment of amnesia should take into account its stage of development.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: NSF Grant 1257923

Title: Origins of behavioral complexity in animals without brains: multiple specialized nerve nets.

Authors: *W. N. FROST[1], J. BERG[2], C. BRANDON[1], T. NOREKIAN[3];
[1]Dept Cell Biol. and Anat., The Chicago Med. Sch., North Chicago, IL; [2]Biol., Lake Forest Col., Lake Forest, IL; [3]Friday Harbor Labs., Friday Harbor, WA

Abstract: Sea anemones have the most primitive nervous system in the animal kingdom. Lacking a brain or CNS, they utilize a nerve net architecture consisting of diffusely distributed neurons, with no ganglia or other centralization. Despite such limited brain power, they have a surprisingly rich behavioral repertoire. For example, the anemone Stomphia displays directed attacks, rapid withdrawals, circular sweeps of the tentacles, rhythmic swimming, and post-swimming righting behaviors. Furthermore, it can distinguish between different predator species, and employs different response strategies to each. Stomphia preferentially attacks the nudibranch Aeolidia papillosa, stinging with its tentacles and then withdrawing, often several times in sequence, to drive the predator away. If unsuccessful, it switches strategies and launches a rhythmic, vigorous escape swim. Stomphia always immediately swims to the seastar Dermasterias imbricata. Strategy switching and multiple sophisticated behaviors. How are these possible with only a "primitive" nerve net organization?
In this study we used confocal imaging of alpha tubulin (neurons) and phalloidin (myoepithelial contractile bundles referred to here as muscle) to examine the nerve net organization of the animal's mesenteries - plate-like structures radiating from the central pharynx to the body wall

that contain the muscles mediating several of these behaviors. One side of each mesentery has a longitudinal retractor muscle that rapidly withdraws the tentacles and shortens the body during defensive withdrawals. The other side has a diagonal parieto-basliar muscle that produces the alternating whole-body bends of the escape swim.
Imaging revealed the presence of several distinct nerve nets associated with the mesenteries. Above each muscle was a diffuse nerve net, with axons running in all directions. Processes from this net then descended to contact a highly organized net of bipolar neurons arrayed in linear fashion along the length of each muscle bundle. A similar dual-net structure was recapitulated for the parieto-basilar muscle on the opposite side of the mesentery. The predominantly connective tissue mesoglea lying between these two nerve net / muscle systems contained a third net of diffuse multipolar neurons, many with long, axon-like processes that appears well situated to mediate communication between the muscle-specific nerve nets on each side of the mesentery. Our results support the idea that in the absence of the nervous system centralization evolved by most other organisms, sea anemones do many things we do through the use of multiple specialized but decentralized nerve nets.

Disclosures: **W.N. Frost:** None. **J. Berg:** None. **C. Brandon:** None. **T. Norekian:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: BB/K018515/1

Title: Role of modulatory interneurons in memory lapses after single-trial reward conditioning

Authors: *D. PRICE, M. CROSSLEY, G. KEMENES, P. R. BENJAMIN, T. NOWOTNY, I. KEMENES;
Neurosci., Univ. of Sussex, Brighton, United Kingdom

Abstract: The feeding system of the pond snail, *Lymnaea stagnalis*, is a well-established model for understanding the neural mechanisms of memory consolidation. Following a single pairing of gamma-nonalactone (GNL), the conditioned stimulus (CS) and sucrose, the unconditioned stimulus (US), a long-term appetitive memory is formed, lasting for weeks. It has previously been shown that a non-synaptic change, a persistent depolarisation of the soma of a modulatory interneuron, the cerebral giant cell (CGC), is involved in the maintenance of long-term memory in *Lymnaea*.

Much less is known about the cellular changes underlying the actual process of memory consolidation. It has been reported in vertebrates and invertebrates that there are brief periods during memory consolidation when the recall of memory is impaired. In *Lymnaea* such periods of vulnerability of the memory trace have been identified both *in vivo* and *in vitro*. To better understand the underlying cellular mechanisms of this process, we recorded the response of two tonically firing modulatory interneurons to the CS, at lapse and non-lapse points.
It is already known that the CGC responds with an increased firing rate when sucrose is applied to the lips. We have now discovered that the CGC can also respond to the CS at a non-lapse point (1 hour), but not at a lapse point (2 hours). This suggests a possible role for the CGC in the conditioned feeding response.
Because the pleuro-buccal interneuron (PlB) exerts strong inhibition on many cell types in the feeding system, including the CGC, it was hypothesised that changes in PlB activity may also be involved in forming the conditioned feeding response. However, no change in the firing activity of PlB was observed during application of the CS in trained individuals.

Disclosures: D. Price: None. **M. Crossley:** None. **G. Kemenes:** None. **P.R. Benjamin:** None. **T. Nowotny:** None. **I. Kemenes:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: RSF Grant 14-25-00072

Title: Cued memory maintenance is different from context memory maintenance

Authors: *P. M. BALABAN, M. ROSHCHIN, A. ZIUZINA, A. VINARSKAYA, A. MALYSHEV;
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Abstract: Retrieval of memory followed by reconsolidation maintains the memory, while retrieval without following reconsolidation results in extinction of memory. In our study in terrestrial snail Helix we made an attempt to describe the conditions in which the retrieval of cued and context memories leads either to extinction or reconsolidation. In the network underlying the withdrawal behavior of this mollusk, sensory neurons, premotor interneurons, motor neurons, and modulatory for this network serotonergic neurons are identified, and recordings from representatives of these groups were made before and after aversive learning *in*

vitro. In the network underlying feeding behavior, the premotor modulatory serotonergic interneurons and motor neurons involved in motor program of feeding are identified, and activity of interneurons was monitored during and after aversive learning. The hypothesis tested in this study concerns the activity of the "reinforcing" serotonergic neurons that is suggested to be the gate condition for the choice between extinction/reconsolidation triggered by memory retrieval: if these serotonergic neurons do not respond during the retrieval, then we will observe the extinction; while if these neurons respond to the CS during memory retrieval, we will observe the reconsolidation phenomenon. Analysis of changes in neural activity after aversive learning *in vitro* showed that modulatory serotonergic neurons of feeding behavior do not demonstrate significant changes, while responses to food in withdrawal behavior premotor interneurons (containing FMRFa) changed qualitatively, from under threshold EPSPs to strong depolarization and spike discharges. Responses to food in identified serotonergic neurons involved in withdrawal (whose activity was shown previously to be necessary and sufficient for reinforcement of long-term synaptic plasticity) also changed from absence of responses before training session to a high-frequency spike discharges to the food stimuli, thus implying that during reactivation of aversive memory the serotonergic cells are activated and are involved in the reconsolidation process. In behavioral experiments it was shown that impairment of functioning of the serotonergic system with the neurotoxin 5,7-DiHT resulted in **complete extinction of context memory** after several reactivations, while **cued memory** to a specific type of food was significantly reduced but still was present. Thus, participation of the “reinforcing” serotonergic neurons in memory retrieval can be the gate condition for the choice between extinction/reconsolidation for context memory but not for cued memory

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Poster

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NSF EAGER Award IOS-1255695

Title: Assessment of alternative strategies for gene knockdown in intact CNS or intact animals of *Aplysia* californica

Authors: M. Y. ZHENG, S. LU, P. SHRESTHA, K. HARRIS, *T. W. ABRAMS;
Pharmacol., Univ. of Maryland Med. Sch., Baltimore, MD

Abstract: With the availability of the nearly complete *Aplysia* transcriptome (Orvis et al, 2015), the number of genes that that are of interest to investigate to understand their contribution to any particular cellular or behavioral process has increased dramatically. The traditional approach for more than two decades of knocking down gene expression in single pre- or postsynaptic neurons by injection of antisense, siRNA or morpholinos now seems extremely limiting. For example, several lines of cellular electrophysiological evidence suggest that the small G protein Arf plays a central role in switching the *Aplysia* sensory neuron-to-motor neuron synaptic connection to a silent state during homosynaptic depression. Five *Aplysia* Arf GEFs have been identified, any of which may participate in this synaptic switch. All five are expressed in the presynaptic sensory neurons. Pharmacological and molecular studies, including dominant negative manipulations, have provided contradictory evidence as to the identity of the GEF responsible for activating Arf at presynaptic release sites. Thus, more efficient knockdown strategies are needed for efficient assessment of the contributions of each of these 5 Arf GEFs, which may act in combination. Based on the experience of Rajasethupathy et al (2009) with penetratin-coupled 2'-O-methyl antisense oligos and our experience with morpholinos (Lin et al, 2010), we are comparing externally applied penetratin-coupled 2'OMe antisense oligos, penetratin-coupled BNA antisense oligos and Vivo-Morpholinos targeted to each of the five Arf GEFs. In preliminary experiments, extracellularly applied Vivo-Morpholino targeted to ApBRAG was highly effective in knocking down BRAG protein expression after 2-3 days of whole ganglia in culture.

Disclosures: M.Y. Zheng: None. **S. Lu:** None. **P. Shrestha:** None. **K. Harris:** None. **T.W. Abrams:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

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NIMH 5T32 MH019524

T32 MH 963314

Title: Presynaptic and postsynaptic effects of *Aplysia* cysteine-rich neurotrophic factor during long-term synaptic facilitation

Authors: *A. ALEXANDRESCU[1], T. J. CAREW[2];
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Abstract: The molecular mechanisms governing long-term memory (LTM) formation are highly conserved across species. One such conserved mechanism is neurotrophic factor signaling which, in addition to being critical for developmental plasticity, is known to contribute to adult synaptic plasticity underlying LTM. While it is known that neurotrophic factors are released locally at synapses in response to neuronal activity involved in LTM formation, their precise pre- and postsynaptic effects remain to be elucidated. The marine mollusk *Aplysia* has proven to be a powerful model system for studying cellular and molecular mechanisms of LTM formation. In *Aplysia*, long-term facilitation (LTF) of monosynaptic connections between identified sensory and motor neurons (SNs and MNs) contributes significantly to LTM for sensitization. Moreover, the SN-MN microcircuit can be reconstituted in culture, offering single cell spatial resolution for the examination of endogenous neurotrophic factor signaling *in vitro*. Our laboratory has identified a novel neurotrophic factor, *Aplysia* cysteine-rich neurotrophic factor (ApCRNF), which shares structural and functional characteristics with mammalian neurotrophic factors (Pu et al. 2014). We previously showed: (i) when paired with subthreshold analog training for sensitization (1 tail nerve shock), exogenous application of recombinant ApCRNF induces ERK/MAPK activation in SNs, a molecular signature required for both LTF and LTM formation, and ii) ApCRNF is released in the medium surrounding the CNS in an activity-dependent manner. Here we studied endogenous ApCRNF signaling in the SN-MN co-culture system. Using immunocytochemical and *in situ* hybridization analyses, we found that both ApCRNF protein and mRNA are localized to the cytoplasm of both SNs and MNs. Furthermore, we found that blocking endogenously released ApCRNF with an anti-ApCRNF antibody, blocks the induction of LTF induced with an activity-dependent training protocol (1 pulse of 5HT in the presence of high KCl). Our findings show that ApCRNF is synthesized in both pre- and postsynaptic neurons and is required for the induction of activity-dependent LTF. Our current studies are focused on determining the critical source of ApCRNF release within the SN-MN microcircuit during the induction of LTF.

Disclosures: **A. Alexandrescu:** None. **T.J. Carew:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: NIMH RO1 MH 041083 grant to TJC

Title: Memory enhancement by IGF2 precedes evolutionary acquisition of IGF2 affinity for the IGF2 receptor

Authors: *N. KUKUSHKIN, T. J. CAREW;
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Abstract: Insulin and related growth factors (IGFs) are important integrators of growth-, energy- and development-related signaling in most metazoans. IGFs generally act through receptor tyrosine kinases (RTKs). Thus, proliferative and pro-survival effects of mammalian IGF2 are mediated predominantly by the IGF1 receptor (IGF1R), an RTK to which both IGF1 and IGF2 have high affinity. IGF2 mediates memory consolidation, enhances memory and prevents forgetting in the rat (Chen et al., 2011). Surprisingly, these effects are not dependent on IGF1R but instead require IGF2R, an additional, non-RTK receptor with preferential affinity to IGF2. The affinity of mammalian IGF2R to IGF2 is widely assumed to be a result of a recent evolutionary gain of function observed exclusively in mammals. In most metazoans, the orthologs of IGF2Rs function in lysosomal sorting. To test if IGF2-mediated enhancement of memory is indeed dependent on its interaction with IGF2R, we studied the effect of human IGF2 on long-term facilitation (LTF) of sensorimotor (SN-MN) synapses in *Aplysia*, whose IGF and IGF2R orthologs lack all residues critical for their interaction in mammals. Strikingly, a 1h pretreatment of the pleural-pedal ganglia (which contain the SNs and MNs) with human IGF2 subsequently enhanced activity-dependent LTF, indicating that IGF2 can act independently of IGF2R.
To study this effect further, we monitored the state of multiple intracellular signaling pathways upon IGF2 treatment. Surprisingly, administration of IGF2 caused an acute repression of phosphorylation by multiple key Ser/Thr kinases (PKA, PKC and Akt) in SN-MN synapses, but especially in the somata of SNs, which are located in the pleural ganglion (separated from the synaptic compartment by the pleural-pedal connective). This repression was abolished (i) by IGF1R inhibitors, and (ii) when the connective was severed before treatment with IGF2, suggesting that retrograde signaling from the synapse to the soma is required to repress Ser/Thr kinases in response to IGF2. Consistent with this view, when the connective was severed prior to IGF2 delivery, IGF2 now produced activation of Ser/Thr kinases in the SN somata. Finally, when ganglia were pretreated with IGF2 for a short period (15 min), subsequent induction of LTF was decreased, consistent with the acute repression of Ser/Thr kinases. Thus, our results reveal the presence of at least two opposing, temporally and spatially specific effects of IGF2 on LTF, and further, suggest the surprising conclusion that the enhancement of LTF by IGFs precedes evolutionary acquisition of IGF2:IGF2R binding.

Disclosures: N. Kukushkin: None. **T.J. Carew:** None.

Poster

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Support: NIH grant SC3GM11118G

Title: cGMP induces a decrease of the intrinsic excitability of *Aplysia* neuron B51 similar to that caused by sensitization training

Authors: A. GOLDNER, M. WAINWRIGHT, *R. MOZZACHIODI;
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Abstract: When *Aplysia* is exposed to aversive stimuli, it exhibits concurrent enhancement of defensive withdrawal reflexes (i.e., sensitization) and suppression of feeding. The duration of these behavioral modifications depends on the amount of training, with a single sensitization trial inducing changes lasting for at least 2 h, but not for 24 h, and four repeated trials inducing long-term changes persisting up to 24 h (Acheampong et al. 2012).
Our lab has recently identified decision-making neuron B51 as a site of plasticity underlying the training-induced suppression of feeding. In particular, sensitization training causes an increase of B51 burst threshold, which is indicative of a decrease of the neuron's intrinsic excitability (Shields-Johnson et al. 2013; Weisz et al. 2013). The goal of this project was to investigate which second-messenger system(s) was responsible for the decrease of B51 intrinsic excitability. Previous findings indicate that elevation of the cytosolic levels of cAMP produced an increase of B51 intrinsic excitability. Therefore, we tested the hypothesis that another cyclic nucleotide, cGMP, could contribute to the decrease of B51excitability induced by sensitization training. Either cGMP or vehicle was iontophoretically injected into B51 using pulses of hyperpolarizing current of -20 nA. To mimic the single-trial sensitization paradigm, one 30-s pulse was used. The membrane properties of B51 (i.e., burst threshold, resting potential and input resistance) were measured prior to and at different time points after injection (15 min, 30 min, 45 min, 1 h, 2 h). Following cGMP injection, B51 burst threshold was significantly increased for up to 45 min, compared to the vehicle-injected cells. B51 burst threshold did not differ statistically between the two groups at the 1 and 2 h time points. No cGMP-induced changes in resting potential or input resistance were observed at any time point.
To mimic the long-term sensitization paradigm, we used a treatment consisting of four 30-s pulses of injected cGMP/vehicle, spaced 30 min apart. B51 membrane properties were measured prior to and 24 h after treatment. Twenty-four hours after treatment, B51 burst threshold was significantly increased in the cGMP group, compared to the vehicle group. No cGMP-induced changes in resting potential or input resistance were observed 24 h after treatment.

These findings indicate that cGMP injection induced a decrease of B51 excitability analogous to that produced by both single-and multiple-trial sensitization training protocols.

Disclosures: **A. Goldner:** None. **M. Wainwright:** None. **R. Mozzachiodi:** None.

Poster

642. Learning and Memory: Invertebrates

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 642.14/KKK30

Topic: H.01. Animal Cognition and Behavior

Support: The University of Texas System Neuroscience and Neurotechnology Research Institute BRAIN Seed Grant

Title: Using directed information to infer functional connectivity in neuronal networks

Authors: ***Z. CAI**[1], C. L. NEVEU[2], J. H. BYRNE[2], B. AAZHANG[1];
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Abstract: The ways in which organization of individual neurons into circuits enables learning, memory and other brain functions is poorly understood. With the advancement in large-scale simultaneous neuronal recording techniques, it is becoming possible to delineate the circuits underlying these recordings, which will also enable us to examine reorganization of these circuits during learning processes. In our experiments, multichannel analog data was obtained from the buccal ganglion of *Aplysia* using voltage sensitive dye (VSD) recording and converted into binary spike trains. Directed information (DI) was used to measure unidirectional influence between neuron pairs, with context tree maximizing (CTM) algorithm serving as the estimator for their stochastic properties. CTM is a data-driven, consistent estimator that automatically finds the best model based on a cost criterion. The analyses also included other biophysical properties such as whether the connections are excitatory or inhibitory and whether their actions were fast or slow. A connectivity diagram with neurons as nodes and connections as edges was then generated and parameters such as the in-/out-degree and the net-flow of a node were calculated. The method was validated using several realistic Hodgkin-Huxley network models where it not only correctly identified the direct connections but also was robust against synapses with nonlinear dynamic properties such as depression and facilitation. The algorithm was applied to VSD recordings. It detected several putative connections, sources, and sinks. This method of augmenting large-scale recording techniques with signal processing tools to construct functional connectomes offers an automated tool to map neural circuits and the ability to capture changes in

synaptic strength as well as the saliency of neurons across different epochs, which will help identify network modifications due to learning and decision-making.

Disclosures: **Z. Cai:** None. **C.L. Neveu:** None. **J.H. Byrne:** None. **B. Aazhang:** None.

Poster

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Program#/Poster#: 642.15/KKK31

Topic: H.01. Animal Cognition and Behavior

Support: R01NS019895

R01NS073974

Title: Mechanism of doxorubicin-mediated persistent ERK activation and enhanced excitability in *Aplysia* sensory neurons

Authors: ***B. COUGHLIN**, H. LAKSHMINARASIMHAN, J. H. BYRNE;
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Abstract: Chemotherapy-induced learning and memory deficits are gaining attention given the increasing number of cancer survivors. Elucidation of the molecular and biophysical perturbations to neurons induced by chemotherapeutic agents may provide insights into mechanisms underlying cognitive deficits. A single, brief exposure of *Aplysia* sensory neurons (SNs) to the anthracycline doxorubicin (DOX) leads to an increase in levels of phosphorylated extracellular signal-regulated kinase (pERK) and p38 mitogen-activated protein kinase (p-p38 MAPK) immediately after treatment. DOX also leads to an increase in SN excitability 24 h after treatment (Liu et al., J. Neurosci. 34:13289, 2014). The increase in pERK persists for 24 h after treatment while the increase in p-p38 MAPK reverses within 2 h of treatment cessation (Lakshminarasimhan et al., 2015). Here, we investigated the persistence of, and mechanism underlying the DOX-induced excitability change and phosphorylation of ERK. DOX treatment resulted in a persistent increase in SN excitability for as long as 48 h after treatment ($p = 0.0001$), accompanied by a decrease in firing threshold ($p = 0.0001$), and an increase in input resistance ($p = 0.001$). U0126, a MEK inhibitor and SB 203580, a p38 MAPK inhibitor, were used to examine the role of ERK and p38 MAPK activation in the persistent increase in excitability. Application of U0126 concurrently with DOX prevented the increase in excitability 24 h after treatment ($p = 0.009$). In contrast, application of SB 203580 during DOX treatment did not affect excitability ($p = 0.7$). Therefore, the long-lasting increase in excitability appears dependent on ERK but not p38

MAPK activation during DOX treatment. Application of U0126 concurrently with DOX did not prevent the persistent activation of ERK ($p = 0.6$). Application of U0126 at a later time point (23 h after the end of DOX treatment) also did not reverse the activation of ERK at 24 h ($p = 0.5$). Application of SB 203580 during DOX treatment did not affect the persistent activation of ERK at 24 h ($p = 0.7$). Collectively, these results suggest that mechanisms other than MEK activation may be required for persistent ERK activation. Further, long-lasting ERK activation and excitability may be mediated by different mechanisms. Persistent enhancement of ERK activation and excitability are similar to changes that occur during learning, suggesting the memory deficits associated with chemobrain may be due to occlusion or saturation of the underlying pathways.

Disclosures: B. Coughlin: None. **H. Lakshminarasimhan:** None. **J.H. Byrne:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: Russell and Diana Hawkins Family Foundation Discovery Fellowship

Title: Combining voltage-sensitive dye (VSD) imaging with extracellular nerve recordings aid in the identification of neurons

Authors: *C. NEVEU[1], R. COSTA[2], R. HOMMA[2], S. NAGAYAMA[2], J. BYRNE[2];
[1]Neurobio. and Anat., The Univ. of Texas Med. Sch. At Houston, Houston, TX; [2]Neurobio. and Anat., UTHealth, Houston, TX

Abstract: VSD imaging can be used to simultaneously record spike activity in 10s-100s of neurons, which provides important advances in our understanding of circuit dynamics. To better understand the physiological role of the neuronal activity it is important to identify the recorded neurons which requires information of not only the size, position, and phasic activity but also the axonal projections of each neuron. Previous research has combined extracellular nerve recording and VSD to characterize neuronal projections of neurons in the feeding network of *Aplysia* (Morton et al. 1991), but did not attempt to identify the recorded neurons. Here we combined VSD recordings and extracellular nerve recordings to identify neurons in order to ascertain their role in feeding motor patterns. A first step in the process was a meta-analysis of previous extensive research characterizing the neurons of the buccal ganglia. Of the 51 neurons that have been studied to date about 14% were local interneurons, 45% send a single ipsilateral projection,

and 18% send projections through multiple nerves. The phasic activity of all neurons was also categorized. 63% were active during either protraction or retraction or both, while only 10% were active during post-retraction. We next characterized the projections and phasic activity of neurons and compared our results with our catalogue in order to identify neurons. Suction electrodes were applied to 7 nerves that connect the buccal ganglia to the periphery (ipsi and contra Bn1,2,3 and ipsi-Rn). Ganglia were stained with RH-155 and the activity of the nerves and VSD were recorded simultaneously for two (2 min) recordings separated by 15 min. A projection was considered to occur if a spike in the nerve followed a spike in the cell with a consistent delay. Using this information from the meta-analysis we identified up to 9 neurons during a single recording. With future experiments we hope to refine our methods to increase our ability to identify neurons and to identify new neurons which have yet to be characterized. We hope to use this technique in the future to track changes in each neuron before and after learning and compare the changes of each neuron between animals.

Disclosures: C. Neveu: None. **R. Costa:** None. **R. Homma:** None. **S. Nagayama:** None. **J. Byrne:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grang 1R15MH107892-01

Title: Transcriptional correlates of forgetting of long-term sensitization memory in *Aplysia* californica

Authors: *R. CALIN-JAGEMAN[1], I. E. CALIN-JAGEMAN[2];
[1]Psychology, [2]Biol., Dominican Univ., River Forest, IL

Abstract: Long-term memories can be maintained indefinitely, but most encoded memories are eventually forgotten. How, then, are long-term memories maintained? And is forgetting due simply to the passive decay of maintenance mechanisms or to an active removal process? We are addressing these questions through the study of long-term sensitization (LTS) of the tail-elicited siphon-withdrawal reflex of *Aplysia californica*. Specifically, we are combining microarray, qPCR, and promoter analysis to characterize the transcriptional networks activated at 1 hour, 1 day, and 1 week after the induction of LTS, time-points which reflect encoding, maintenance, and forgetting, respectively. Our approach leverages several strategies to ensure high sensitivity

and replicability: adequate sample sizes, confirmation in independent samples, within-subjects comparisons, and a focus on a single, homogenous set of neurons specifically involved in the expression of LTS memory. Our results show that initial encoding produces strong and rapid regulation of about 80 transcripts. This initial transcriptional response to LTS training ramifies during maintenance, with over 600 transcripts strongly regulated 1 day after induction. As forgetting sets in, essentially all of these maintenance-related transcriptional changes fade away, but overlaid on this decay process there seems to be an activation of a very small set of putative forgetting-related transcripts. We are now seeking to identify promoter motifs that help coordinate the transcriptional responses related to encoding, maintenance, and forgetting.

Disclosures: R. Calin-Jageman: None. **I.E. Calin-Jageman:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: KAKENHI 26560223

Title: Learning effect on the response time of S-cluster of *Aplysia* to taste observed by voltage sensitive dye imaging

Authors: *T. YANAGI[1], Y. YOSHIMI[1], T. NAGAHAMA[2];
[1]Dept. Applied Chem., Shibaura Inst. of Technol., Tokyo, Japan; [2]Dept. Biophysics, Toho Univ., Tokyo, Japan

Abstract: Taste is an important sense for determining whether the intaken food to be ingested or rejected for survival. A marine gastropod *Aplysia* has large neurons and shows clear taste preferences, which is modified by the experience and learning. We attempted to analyze the neural network which generates ingestive or rejective response by using fluorescent voltage sensitive dye (VSD) imaging. Buccal mass and buccal ganglion was isolated with retaining connection between them via buccal nerves. The neurons in the ganglion were stained with VSD (Di-4-ANEPPS) in the presence of tetraethylammonium (TEA) chloride to prolong the action potential. Then the responses of the fluorescent image were monitored after administration of preferred ($_L$-asparagine, *Undaria*, *Porphyra*) or unpreferred taste (distilled water, $_L$-aspartic acids) to radula inside the buccal mass. VSD imaging revealed that theVSD imaging revealed that the S cluster neurons responded to unpreferred taste more rapidly (latency of *ca.* 2 s) than preferred one (latency of *ca.* 4 s). In the present experiments *Aplysia* learned to reject $_L$-

asparagine by an electrical shock during tasting it. Then the latency of the S-cluster response to the L-asparagine in the trained *Aplysia* was shortened to 2 s like that to the unpreferred tastes. Conversely *Aplysia* learned to inject L-asparatic acid by the subsequent rewarding with *Porphyra* extract. Then the latency of the S-cluster response to the L-asparatic acid in the trained Aplysia was prolonged to 4 s like that to the preferred tastes. These results indicate that the peripheral neural circuit probably decides the behavior responding to the taste before the S-cluster neurons receive the signals of the taste.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: NSF 00123024

NIH 00095923

Title: Epitranscriptomic landscape of single neurons: Insights in the memory mechanisms

Authors: *A. B. KOHN[1], M. BASANTA SANCHEZ[2], L. L. MOROZ[3,4];
[1]Universtiy of Florida Whitney Lab., Saint Augustine, FL; [2]Univ. of Albany, The RNA Inst., Albany, NY; [3]Univ. of Florida, Neuroscience and McKnight Brain Institute, FL; [4]Whitney Lab. for Marine Biosci., St Augustine, FL

Abstract: Post-transcriptional changes in RNA have the potential to influence the epigenetic landscape. There are over a hundred RNA modifications. These chemical changes to nucleotides do not alter the sequence of RNA but can alter gene expression and has recently been described as part of a so-called epitranscriptome. Methylation of adenosine to N6-methyladenine (m6A) is the most prevalent internal modification on mRNA and long non-coding RNA with up to 20% of the human mRNA methylated. However, little is known about biological roles of RNA modifications. Here, we used *Aplysia californica*, a prominent model organisms to study learning and memory, and directly chemically characterized the scope of the epitranscriptome focusing at the level of individual functionally identified neurons. We determine that 4% of the total RNA was methylated in Aplysia. RNA-seq and computation analysis of the *Aplysia* genome shows that all the enzyme families involved in RNA methylation/demethylation are present in varying amounts from developmental states to single neurons following neuroplasticity tests. For the first

time RNA modifications were identified and quantified in single neurons using ultrasensitive mass spectrometry (MS) in combination ultra-high performance liquid chromatography. Different cholinergic neurons in *Aplysia* showed different and distinct RNA modifications. In addition to the RNA modification, we also discover cell- and tissue specific RNA editing in *Aplysia*. This adds another layer to the epigenetic regulation forming a complex epitranscriptomic landscape. RNA editing is a process of targeted alterations of nucleotides in all types of RNA molecules (e.g., rRNA, tRNA, mRNA, and miRNA). As a result, the transcriptional output differs from its genomic DNA template. RNA editing can be defined both by biochemical mechanisms and by enzymes that perform these reactions. We found full a repertoire of predicted RNA-editing enzymes in the genome of *Aplysia* including several expansions of enzyme families compared to what has been described in mammals. In summary our data indicate that both single-cell transcriptome and epitranscriptomes are unique features of identified neurons providing novel insights into genomic bases of neuronal individuality and plasticity.

Disclosures: **A.B. Kohn:** None. **M. Basanta Sanchez:** None. **L.L. Moroz:** None.

Poster

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Support: HHMI

NIH grant NS083690

Title: *Aplysia* neurotrophin acts as a presynaptic autocrine signal to regulate protein synthesis in the presynaptic neuron during the induction of intermediate-term facilitation produced by 5HT at *Aplysia* sensorimotor neuron synapses

Authors: ***I. JIN**[1], H. UDO[3], S. KASSABOV[1], R. NICOLLS[2], H. ZHU[1], L. KIM[1], E. R. KANDEL[1], R. D. HAWKINS[1];
[1]Dept. of Neurosci., [2]Pthology and Cell Biol., Columbia Univ., New York, NY; [3]Dept. of Biol., Kyushu Univ., Fukuoka, Japan

Abstract: The transition from short-term facilitation (STF) to intermediate-term facilitation (ITF) produced by 5-HT at sensorimotor neuron synapses of *Aplysia* is characterized by two major changes: the recruitment of postsynaptic as well as presynaptic mechanisms of facilitation

and the activation of protein synthesis in both the presynaptic and postsynaptic neurons (Jin et al., 2011). We found that spontaneous glutamate release from the presynaptic neuron acts as an anterograde signal to recruit postsynaptic mechanisms during the induction of ITF (Jin et al., 2012a, b). *Aplysia* neurotrophin (ApNT) released from the presynaptic neuron also contributes to the recruitment of postsynaptic mechanisms by (1) acting as an autocrine signal that forms a positive feedback loop with its receptor (ApTrk receptor) and PKA in the presynaptic neuron, thus enhancing spontaneous release of glutamate and ApNT (permissive role), and (2) acting as an anterograde signal to activate ApTrk receptors on the postsynaptic neuron during the induction of the ITF (Jin et al., Soc. Neurosci. Abstr. 2014, 2015). We have now asked, how is protein synthesis activated during the transition to ITF? We find that the positive feedback loop also regulates protein synthesis in the presynaptic neuron through activation of presynaptic ApTrk receptors. In vertebrates, activation of the TrkB receptor by its ligand BDNF induces protein synthesis via two downstream signaling pathways, PI3K/Akt and Ras/ERK (Robinet and Pellerin, 2010). We found that activation of presynaptic ApTrk receptors in *Aplysia* also increased the amount of p-AKT and p-ERK1/2 in the presynaptic neuron ($p< 0.001$ for both) and more specifically, the activation increased the amount of phospho-eukaryotic translation initiation factor 4E (p-eIF4E), the rate-limiting component of the eukaryotic translation apparatus ($p< 0.001$). Consistent with these results bath application of the protein synthesis inhibitor anisomycin reduced the increase in mini frequency produced either by mature ApNT or by activation of the presynaptic ApTrk receptor ($p< 0.05$ for both). These results suggest that the positive feedback loop in the presynaptic neuron plays two major roles during the induction of the ITF: (1) it contributes to the recruitment of postsynaptic mechanisms and (2) it regulates protein synthesis in the presynaptic neuron. The positive feedback loop may also play an important role in the transition to long-term facilitation (LTF) by increasing the levels of presynaptic PKA and MAPK sufficient for translocation of these two kinases into the nucleus, where they activate transcription during the induction of LTF (Martin et al., 1997).

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Poster

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Iris and Junming Le Foundation

Title: *Drosophila* SLC22A transporter is a memory suppressor gene that influences cholinergic neurotransmission to the mushroom bodies

Authors: *Y. GAI, Z. LIU, I. CERVANTES-SANDOVAL, R. DAVIS;
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Abstract: The mechanisms that constrain memory formation are of special interest because they provide insights into the brain's memory management systems and potential avenues for correcting cognitive disorders. RNAi knockdown in the *Drosophila* mushroom body neurons (MBn) of a newly discovered memory suppressor gene, Solute Carrier DmSLC22A, a member of the organic cation transporter family, enhances olfactory memory expression, while overexpression inhibits it. Immunohistochemistry coupled with super-resolution microscopy revealed that the protein localizes to the dendrites of the MBn, surrounding the presynaptic terminals of cholinergic afferent fibers from projection neurons (Pn). Transport assays using expression of DmSLC22A in HEK 293 cells and *Drosophila* primary neurons show that this plasma membrane protein transports cholinergic compounds with the highest affinity among a battery of in vitro substrates that were tested. Feeding flies choline enhanced memory; an effect blocked by overexpression of the transporter in the MBn. Functional imaging indicates that DmSLC22A knockdown enhances the calcium responses at the MBn calyx due to odor-triggered excitation of cholinergic Pn, while overexpression restrains them. Furthermore, genetic manipulation of cholinergic neurotransmission at Pn:MBn synapses revealed that overexpression of DmSLC22A in MBn suppresses the enhanced memory expression due to AChE knockdown in the Pn. The combined data show that DmSLC22A is a memory suppressor protein that limits memory formation by helping to terminate cholinergic neurotransmission at the Pn/MBn synapse.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant 2R37NS19904

NIH Grant 2R01NS05235

Title: Scribble scaffolds a signalosome for active forgetting

Authors: *I. CERVANTES-SANDOVAL[1], M. CHAKRABORTY[2], C. M. MACMULLEN[2], R. L. DAVIS[2];
[1]Neurosci., The Scripps Res. Inst., Jupiter, FL; [2]Neurosci., The Scripps Res. Inst., Jupiter, FL

Abstract: Forgetting, one part of the brain's memory management system, provides balance to the encoding and the consolidation of new information by removing unused or unwanted memories or by suppressing their expression. Recent studies identified the small G-protein, Rac1, as a key player in the *Drosophila* mushroom bodies neurons (MBn) for, active forgetting. Subsequently it was discovered that a few dopaminergic neurons (DAn) that innervate the MBn mediate forgetting. Here we show that Scribble, a scaffolding protein known primarily for its role as a cell polarity determinant, piece together the intracellular molecular machinery that constitutes the signal for normal forgetting. Knocking down *scribble* expression in either MBn or DAn impairs normal memory loss. Scribble interacts physically and genetically with Rac1, Pak3 and Cofilin within MBn, nucleating a forgetting signalosome that is downstream of dopaminergic inputs that regulate forgetting. These results bind unrelated molecular players in active forgetting into a single signaling pathway: Dopamine>Dopamine Receptor> Scribble> Rac> Cofilin.

Disclosures: I. Cervantes-Sandoval: None. **M. Chakraborty:** None. **C.M. MacMullen:** None. **R.L. Davis:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Title: The role of presynaptic D2 receptor in dopamine neuronal excitability and synaptic release underlying *Drosophila* larval olfactory associative learning

Authors: C. QI[1], *D. LEE[2];
[1]Biol. Sci., [2]Ohio Univ., Athens, OH

Abstract: Dopamine (DA) plays an important role in modulating neuronal signaling and cognition. Therefore, understanding the molecular properties of DA signaling is an essential step in decoding the sub-cellular physiology underlying associative learning. The physiological

functions of DA are mediated through G-protein coupled receptors. Generally, DA receptors can be divided into two subfamilies: excitatory D1- and inhibitory D2-like receptors. In Drosophila melanogaster, it has been shown that postsynaptic D1 receptors are involved in associative olfactory learning both in adults and larvae. However, it is expected that inhibitory D2 autoreceptors modulating synaptic DA release also play a crucial role in olfactory learning. Currently, little is known about the role of presynaptic D2 receptors (DD2R) in Drosophila associative learning. In this study, we wanted to examine how presynaptic DD2R regulates functional properties of DA neurons and thus olfactory learning in Drosophila.First, we examined the role of presynaptic DD2Rs in Drosophila larval associative learning. In the olfactory conditioning, an odor pentyl acetate was used as a conditional stimulus (CS), and 1M sucrose (appetitive) or 0.1 percent quinine (aversive) was used as an unconditional stimulus (US). Late third instar larvae (92-96h) were collected and trained for 30 min, then their performance towards CS was tested and compared to the control group with no US. Using a UAS-DD2R-RNAi line crossed with a DA-specific driver (TH-Gal4), we observed that knockdown of presynaptic DD2R impaired both appetitive and aversive learning in F1 larvae. As DD2R is an inhibitory G-protein coupled receptor, we hypothesized that, DD2R autoreceptor mediates olfactory learning through down-regulation of DA neuronal excitability and presynaptic DA release. In a well-established Drosophila primary neuronal culture, action potential (AP) frequency in GFP-marked DA neurons can be measured using the whole-cell patch technique. Our results show that the DA neuronal AP frequency was inhibited by a D2 agonist quinpirole (10 microM), indicating an inhibitory role of DD2R in DA neuronal excitability. Further, we were able to measure distinct DA synaptic currents in GFP-marked DA neuronal processes using amperometry method with a carbon-fiber electrode. We are currently examining the role of DD2R autoreceptors in DA neuronal excitability and synaptic DA release using genetic and pharmacological approaches. We also plan to study the role of presynaptic D2 receptors in DA neuronal excitability and synaptic DA release in the intact larval brain.

Disclosures: **C. Qi:** None. **D. Lee:** None.

Poster

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Program#/Poster#: 642.24/KKK40

Topic: H.01. Animal Cognition and Behavior

Title: A fine-grained analysis of dopamine as a reinforcer of operant behavior in *Drosophila*

Authors: *C. C. ROHRSEN[1], B. DE BIVORT[3], B. BREMBS[2];
[2]Developmental Biol. and Neurogenetics, [1]Regensburg Univ., Regensburg, Germany;
[3]Organismic and Evolutionary Biol., Harvard Univ., Cambridge, MA

Abstract: Dopamine (DA) is known to be involved in assigning value to events, in both in vertebrates and in invertebrates. For instance, in Drosophila, DA neurons are known to play an essential role in associative conditioning. The valence of learning depends on the DA cluster involved: whereas the protocerebral anterior medial cluster (PAM) mediates appetitive conditioning, the protocerebral posterior lateral cluster 1 (PPL1) mediates aversive conditioning. Studies in the fruit fly have focused in these two clusters because the neurons in these clusters project to the mushroom body, an essential brain structure for olfactory classical conditioning. It is currently not known to which extent DA neurons are involved in modulating ongoing action selection and choice behavior. For assessing the role of different sub-populations of DA neurons in mediating valence, we took advantage of flies expressing the optogenetic channel Chrimson under different DA promoters. These transgenic flies were tested in different decision-making paradigms where reinforcement is contingent on their ongoing choices. Our results highlight the potential, but also the technical hurdles which need to be surmounted to unleash the potential of this optogenetic technique in behaving animals. In addition we construct a model with estimated valences for each of the DA clusters to explain the fly behavior under these conditions. Taken together, the results suggest that there may not be a clear relationship between which neurons mediate, say, appetitive classical conditioning and which neurons can functionally replace reward or punishment in operant situations.

Disclosures: C.C. Rohrsen: None. **B. De Bivort:** None. **B. Brembs:** None.

Poster

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Topic: G.02. Motivation

Support: NIH Grant GM067310

Title: Hormonal convergence in regulation of *Drosophila* long-term memory

Authors: *S.-S. LEE, M. E. ADAMS;
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Abstract: During insect development, the endocrine peptide ecdysis triggering hormone (ETH) regulates ecdysis (cuticle shedding) by orchestrating sequential activity in downstream peptidergic ensembles. Although ETH signaling molecules persist during adulthood in *Drosophila*, their functional roles have yet to be revealed. Here we show that ETH is required for *Drosophila* long-term memory (LTM) performance through direct and indirect actions on the mushroom body. ETH promotes juvenile hormone (JH) synthesis during adulthood. Suppression of ETH and JH signaling in adult males impairs LTM. In particular, RNA knockdown of either ETH or JH receptors in mushroom body neurons compromises LTM, revealing a hormonal convergence in the same functional subdivision to regulate memory formation. Increased release of ETH not only improves LTM performance by the traditional conditioning, but also reduces the training period necessary for memory formation. ETH enhancement also leads to longer-lasting memory. Our findings suggest a model for convergence of ETH and JH signaling in *Drosophila* LTM formation that parallels previously reported instances in mammalian models.

Disclosures: S. Lee: None. **M.E. Adams:** None.

Poster

642. Learning and Memory: Invertebrates

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant DK107900

Title: Generating a new fly model for GALT-loss

Authors: V. BAGGETT[1], J. FRIDOVICH-KEIL[3], *T. ZARS[2];
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Abstract: Galactosemia is a rare genetic disorder that affects 1 in 50,000 newborns and can lead to infant death if dietary galactose is not removed immediately. Three types of galactosemia have been described, one for each of the three enzymes within the galactose metabolism pathway (the Leloir pathway). The most prevalent - type 1 - or loss of function of galactose-1-phosphate uridyltransferase (GALT) gene is known as classic galactosemia (CG). Even with removal of dietary galactose and alleviation of acute symptoms, chronic disease states are associated with neural dysfunction and behavioral changes. Models of GALT loss have been established in yeast, mouse, and *Drosophila.* While each of these models has their limits, experiments demonstrate oxidative stress and glycosylation defects as primary factors responsible for neuronal changes.

With this, we hypothesize that drastic cellular changes with loss of GALT will result in behavioral changes. Preliminary results with a dGALT10 mutant fly show an abnormal latency to re-explore after training in place learning. Due to the nature of the dGALT10 allele, we are generating a new GALT-null mutant in which we specifically excise the catalytic domain. We will test for cellular and behavioral changes in this new model, which will provide targets for treatments in CG patients.

Disclosures: V. Baggett: None. **J. Fridovich-Keil:** None. **T. Zars:** None.

Poster

642. Learning and Memory: Invertebrates

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Topic: H.01. Animal Cognition and Behavior

Support: UCSD Frontiers of Innovation Scholars Program

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Title: Learning-based decision-making behavior of fruit flies

Authors: *R. SUN[1,2], R. GREENSPAN[3];
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Abstract: Decision-making, behaviorally, is a process of choosing between at least two options. Using a novel behavior setup, we study freely-moving flies' learning the and decision-making process and mechanisms underlying such processes in the fruit fly, Drosophila melanogaster. Can flies make decisions against their innate behaviors? To answer this question, I built a novel behavior apparatus in which single flies experienced heat stress when and only when it was moving. Flies have an innate heat avoidance behavior. When they experience uncomfortable heat, they increase their locomotion activity. In this new paradigm, flies were trained to stop walking when encountering heat. After two spaced training sessions, flies showed significant inhibition of their locomotion activity compared to controls.

Disclosures: R. Sun: None. **R. Greenspan:** None.

Poster

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Title: Cdc42-dependent forgetting regulates repetition effect in prolonging memory retention

Authors: *X. ZHANG, Q. LI, L. WANG, Y. ZHONG;
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Abstract: Repeated learning is a daily-used and powerful way to improve memory. A fundamental question is how multiple learning trials add up to improve memory. While the major studies of such a repetition effect so far all emphasize the strengthening of memory formation, the current study reveals a novel molecular mechanism through suppression of forgetting. We find that single-session training leads to formation of anesthesia-resistant memory (ARM) and then activation of the small G protein Cdc42 to cause decay or forgetting of ARM within 24 hrs. Repetition suppresses the activation of Cdc42-dependent forgetting, instead of enhancing ARM formation, leading to prolonged ARM. Consistently, inhibition of Cdc42 activity through genetic manipulation mimicked the repetition effect while repetition-induced ARM improvement is abolished by elevated Cdc42 activity. Thus, only the first session in repetitive training contributes to ARM formation, while the subsequent sessions are not devoted to acquiring information, but to inhibiting forgetting.

Disclosures: **X. Zhang:** None. **Q. Li:** None. **L. Wang:** None. **Y. Zhong:** None.

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Topic: H.01. Animal Cognition and Behavior

Title: Mushroom body-Dopaminergic recurrent network in *Drosophila*

Authors: *Y. JIANG, S. KUNES;
Harvard Univ., Cambridge, MA

Abstract: Dopamine has been established as a simple reinforcement signal in aversive and appetitive memory in fruit flies. Yet in mammalian systems, dopamine acts in accord with the difference of an experience from expectation (ie. prediction error). Specific dopamine neurons (DANs) can be activated by an aversive stimulus such as electric shock or reward stimulus such as sugar. We have found a population of DANs that not only represent Unconditioned Stimuli (US) but also respond to Conditioned Stimuli (CS) as a result of conditioning. We refer to them as *plastic DANs* (pDANs). In live imaging experiments, animals were presented with US and CS stimuli while neuronal Ca2+ activity was monitored. We found that some DANs that innervate the mushroom body (MB) lobes that respond to both US (shock) and CS (odor), including gamma3, gamma4,beta'1 and beta'2 DANs. Those DANs gradually generate differential responses to the CS+/CS-. For example, the gamma4 DAN diminishes its response to CS+ and increases/maintains its response to CS- during and after training with coincident US presentation. To further understand the functions of these special pDANs, we sought to uncover a collection of neurons that might work together with them in a network. We identified more mushroom body output neurons (MBONs) required for aversive memory and novel classes of plastic odor-responsive neurons using the assay mentioned above. We found that MBONs, as expected, as well as a novel class of interneuron (Recurrent Loop Neurons,RLNs) feedback onto pDANs. Activation or inhibition of certain MBONs or RLNs can activate or inhibit pDANs. In addition, we found that some RLNs oscillate or burst in a certain way similar to pDANs. Considering that MBONs can be activated by odor-responsive intrinsic MB cells and that pDANs release dopamine onto these neurons, we propose a recurrent neural network model involving both intrinsic and extrinsic MB neurons. Our behavioral data indicates that this neural network is required to drive memory stabilization in Drosophila. In addition, training flies by standard electric shock paradigm not only induces aversion to CS+ but also attraction to CS-. We show that CS- activates appetitive DANs during aversive training, which induces attraction to CS-. Attraction to CS- further biases fly's choices away from aversive CS+.

Disclosures: **Y. Jiang:** None. **S. Kunes:** None.

Poster

642. Learning and Memory: Invertebrates

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Program#/Poster#: 642.30/KKK46

Topic: G.01. Appetitive and Aversive Learning

Title: Characterization of a Classical Conditioning protocol using discrete stimuli to uncover dynamics of connectome plasticity in *C. elegans*

Authors: L. K. ALFILER[1], A. H. BLACK[1], *J. K. ROSE[2];
[1]Behavioral Neurosci. Program, [2]Psychology, Western Washington Univ., Bellingham, WA

Abstract: The well-described nervous system and connectome of *C. elegans* allows for the study of plasticity mechanisms within identified neural networks and has led to the discovery of several mechanisms of learning and memory. The goal of the current work is to establish a Classical Conditioning protocol employing discrete stimuli to allow for precise and controlled stimulus delivery, and thus greater manipulation of training parameters to reveal both dynamics and mechanisms of connectome plasticity. Previously, our lab has paired a blue light (~480 nm; unconditioned stimulus) with a mild mechanosensory vibration (Vib; neutral stimulus) resulting in increased responsiveness to the Vib stimulus alone, considered to have resulted from the convergence of neuron activation signals likely at the command interneurons. When the number of pairings was increased to five, each pairing presented one minute apart, a more robust increase in responsiveness to the Vib stimulus alone was measured. To further probe the optimal training parameters for conditioning, the intertrial interval was increased to 5 minutes and extinction for this learning was explored. As well, the potential for spaced training to increase retention duration was also tested by delivering multiple pairings during distinct training periods. Finally, earlier studies in our lab have demonstrated the *unc-43*(gk452) mutant strain (ortholog to $CaMKII_{\gamma}$) shows a deficit in associative chemotaxis, therefore the role of this molecule in blue light/Vib network plasticity was also investigated. By introducing and characterizing a training protocol in *C. elegans*, whereby extent of associative conditioning can be prescribed, current and future studies aim to identify and examine mutants of additional signalling genes involved in network dynamics and learning.

Disclosures: **L.K. Alfiler:** None. **A.H. Black:** None. **J.K. Rose:** None.

Poster

643. Neural Circuits for Timing, Temporal Processing, and Sequences

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Program#/Poster#: 643.01/KKK47

Topic: H.01. Animal Cognition and Behavior

Title: Differential changes in rate and ensemble temporal dynamics during sleep following de novo spatial experience

Authors: *G. DRAGOI, J. SIBILLE;
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Abstract: Ensembles of hippocampal CA1 place cells fire sequentially along the trajectory of the animal. The sequence of place cells expressed during run is replayed during post-run sleep, a phenomenon thought to represent the memory of the run experience. However, the sequence of place cell firing during run is also correlated with temporal sequences occurring during the pre-run sleep periods, a phenomenon called preplay. Preplay is believed to represent the pre-configuration of the hippocampal network into cellular assemblies and to facilitate the rapid encoding of novel spatial information in naïve animals. Activation of place cells during run in novel spatial environments results in stronger Bayesian decoding of animal's trajectory during post-run (replay) compared with pre-run sleep (preplay) in previously trained rats. In contrast, in naïve mice, the incidence of temporal sequences of neuronal firing that are correlated with place cell sequences during run, calculated using template-matching procedure, is similar in the post-run (replay) and pre-run sleep (preplay). To further understand the effect of run experience on the hippocampal network dynamics during sleep and explain the conflicting difference between decoding and template matching results, we performed recording of ensembles of CA1 neurons from two independent groups of adult naïve rats (group M, n=3; group Y, n=3). For each pre-run and post-run session, using template matching method, each temporal sequence event during sleep was correlated with the place cell sequences during run (two templates/animal; data correlations) and the results were compared with correlations between the temporal sequence event and 500-times shuffled place cell sequences (1,000 shuffles/event, using 2 templates; shuffle correlations). The results for the two independent groups of rats were remarkably similar. Both pre-run and post-run events were stronger correlated with the run templates (data correlations) compared with the shuffle templates (shuffle correlations) and, for both groups, pre-run and post-run correlations were not different. The incidences of significant preplay and replay events (template matching) were also not different. In contrast, for both groups, the average firing rates of pyramidal neurons were higher in the post-run compared with the pre-run sleep, which can explain the stronger Bayesian decoding of animal's run trajectory during post-run (replay) compared with pre-run sleep (preplay). We propose that specific novel spatial

experiences induce firing rate changes in the hippocampal network during sleep, which are integrated into stable, homeostatic ensemble temporal dynamics.

Disclosures: **G. Dragoi:** None. **J. Sibille:** None.

Poster

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Title: Dynamic resonance controls oscillation frequency in optogenetically induced sharp-wave ripples.

Authors: *D. C. KLORIG, G. ALBERTO, D. GODWIN;
Neurosci. Program, Wake Forest Hlth. Sci., Winston Salem, NC

Abstract: Neural processing occurs in parallel across circuits of varying complexity and size. Multiple streams of information are coordinated in time; yet, the brain lacks a fixed global clock. Variable myelination allows for fine tuning of transmission speeds and processing time, however myelination is relatively fixed, at least on time scales relevant to brain function. At these time scales, oscillatory dynamics in neural populations are thought to temporally coordinate activity. We describe a simple mechanism, governed by the interplay of excitatory and inhibitory processes, by which oscillatory activity varies over a range of frequencies. Using optogenetic light ramp stimuli to induce, rather than evoke, ripple frequency oscillations in area CA1 of freely moving mice, we characterized the dependence of oscillation frequency on the magnitude of the depolarizing input to pyramidal cell populations and using pharmacological manipulation, on the time constant of pacing GABAergic inhibition. A spiking network model is presented that captures the experimentally observed dynamics. As predicted by the model, CA1 exhibits locally

governed resonance in the ripple frequency range that changes with the magnitude of the depolarizing input to the excitatory population. We propose that such an arrangement could allow the frequency of the oscillation, and therefore the temporal window of integration, to vary depending on the number or magnitude of active synaptic inputs, while holding input selectivity constant. Activity involving larger populations of cells, by virtue of the dynamics of E-I networks, would have increased processing speeds and shorter integration times. Such a mechanism could instantiate a dynamic time base that varies depending on the size of the active population, thus keeping overall integration time constant and allowing for efficient parallel processing.

Disclosures: **D.C. Klorig:** None. **G. Alberto:** None. **D. Godwin:** None.

Poster

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Support: NSF Grant IOS-1150292

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Title: Using DREADDs to compare the effects of inactivating CA3 versus the CA3-CA1 projection on the memory for sequences of events.

Authors: ***J. S. A. ASEM**[1], M. H. KASSIR[1], N. B. MIRZA[1], N. N. CHMIELEWSKI[1], G. A. ELIAS[1], C.-W. NG[1], T. A. ALLEN[2], N. J. FORTIN[1];
[1]Neurobio. and Behavior, Univ. of California (Irvine), Irvine, CA; [2]Psychology, Florida Intl. Univ., Miami, FL

Abstract: The ability to temporally organize information is fundamental to many perceptual, cognitive, and motor processes. Temporal organization is also a defining feature of episodic memory, as the memory for individual events includes information about when they occurred. In particular, the ability to remember sequential relationships among events or stimuli is shared by a variety of species including humans, non-human primates, and rodents. Our laboratory has recently developed and validated a cross-species paradigm to test nonspatial sequential memory; recent work using this approach has demonstrated that the hippocampus plays a critical role in this ability. Notably, whole hippocampal inactivations produce substantial deficits in the memory

for a sequence of items (Allen et al., in prep.), and electrophysiological recordings show that dorsal CA1 neurons discriminate the temporal context in which items are presented (Allen et al., 2016). In particular, such neurons fire differentially to odors depending on whether they were presented in sequence or out of sequence. Here, we aim to further investigate the specific hippocampal circuitry supporting this form of sequence memory and, specifically, the role of subregion CA3. Computational models suggest that CA3 may be particularly well-suited to code for sequential relationships among items because of its high degree of recurrent connections. To test this hypothesis, we compared the effects of whole CA3 inactivation to specific CA3-CA1 projection inactivation using designer receptors exclusively activated by designer drugs (DREADDs). Subjects consisted of ten Long-Evans rats, who underwent bilateral infusions of either AAV9 (experimental virus) or rAAV8 (control virus) into dorsal CA3, as well as bilateral implantation of guide cannulae into dorsal CA1. After reaching criterion in the olfactory sequence memory task, neuronal inactivation is induced by clozapine-N-oxide (CNO) injections, with phosphate-buffered saline (PBS) as a control. Systemic CNO injections inactivate the entire CA3 subregion, whereas local infusions specifically block CA3-CA1 communication. This approach allows for testing the general contribution of CA3 as well as the specific role of the CA3-CA1 pathway in the ability to remember a sequence of items.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: NSF IOS-1150292

NSF BCS-1439267

Title: Nonspatial sequence coding varies along the CA1 transverse axis

Authors: ***C. NG**[1], G. A. ELIAS[1], J. S. A. ASEM[1], T. A. ALLEN[2], N. J. FORTIN[1]; [1]Neurobio. & Behavior, Univ. of California, Irvine, CA; [2]Psychology, Florida Intl. Univ., Miami, FL

Abstract: The hippocampus plays a critical role in the memory for sequences of events, a defining feature of episodic memory. To shed light on the fundamental mechanisms supporting

this capacity, we recently recorded neural activity in CA1 as rats performed a nonspatial odor sequence memory task (Allen et al., 2016, *JNeurosci*). Briefly, the task involves the presentation of repeated sequences of odors at a single port and requires rats to identify each item as "in sequence" or "out of sequence". Our main finding was that, while the animals' location and behavior remained constant, a proportion of CA1 neurons fired differentially to odors depending on whether they were presented in or out of sequence (sequence cells). Here, we further examined if such sequence coding varied along the distal-to-proximal axis of the dorsal CA1 region, as suggested by recent anatomical and electrophysiological evidence that odor information may be more strongly represented in the distal segment (compared to the proximal segment, which may predominantly represent spatial information; Igarashi et al., 2014). Recorded neurons were grouped according to their targeted section of CA1, from distal (section 1: ~1 mm ML) to proximal (section 4; ~5mm ML). Preliminary results indicate that while sequence cell coding was observed across the distal-to-proximal extent of CA1 we recorded, it was significantly higher near the transition zone between distal and proximal regions (section 2; ~2.5mm ML). More specifically, in that particular segment of CA1, we observed an increase in the proportion of sequence cells, as well as an overall increase in the magnitude of sequence cell coding and in sequential information content (measured across all cells in that segment). These results suggest nonspatial sequence memory coding is not uniformly distributed along the transverse axis of CA1, and that this distribution does not simply follow the expected gradient based on the modality of the information.

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Poster

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Title: Characterizing the time course of radiation-induced brain changes in memory performance and network activity in rats

Authors: *N. N. CHMIELEWSKI, C.-W. NG, J. S. A. ASEM, G. A. ELIAS, C. L. LIMOLI, N. J. FORTIN;
Univ. of California, Irvine, Irvine, CA

Abstract: Cranial radiation-induced debilitating effects on cognition have long been observed in surviving brain cancer patients. However, the fundamental neural mechanisms causing these persistent cognitive decrements are still unclear. To address this issue, we used a rat model to quantify the progression of memory deficits induced by clinically-relevant doses of X-ray and examine the corresponding changes in neural activity. In the first experiment, rats were trained on a nonspatial sequence memory task, which was previously shown to have strong behavioral parallels in rats and humans (Allen et al., 2014) and to depend on similar hippocampal and prefrontal cortical circuits in both species (Allen et al., in prep; Boucquey et al., submitted). Briefly, the task involves repeated presentations of a sequence of four odors at a single odor port and requires rats to identify each odor as "in sequence" or "out of sequence." After reaching criterion, rats underwent cranial radiation treatment and task performance was assessed over a period of 10 weeks. Performance on the task decreased 6 weeks after irradiation, consistent with past findings of radiation-induced cognitive decrements in common rodent behavioral paradigms associated with hippocampal and prefrontal function. In the second experiment, arrays of recording electrodes were implanted post-irradiation into task-critical and control brain regions. Local field potentials (LFP) were then recorded as rats ran along a two-dimensional track. Compared to controls, irradiated rats began showing changes in LFP power in radiosensitive hippocampal CA1 and CA3 subregions as early as 3 weeks post-irradiation (decreased power for frequencies < 8 Hz, increased power for 8-10 Hz). These data suggest that this approach can detect radiation-induced changes in neural activity prior to cognitive sequelae. Ongoing efforts are aimed at elucidating the cause of these changes in LFP activity and establishing a link with the subsequent emergence of behavioral impairments.

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Poster

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NIDCD T32 DC010775 to G.A. Elias

Title: Distinct contributions of hippocampal, prefrontal, perirhinal and nucleus reuniens regions to the memory for sequences of events

Authors: *N. J. FORTIN[1], J. A. ASEM[1], C.-W. NG[1], C. R. QUIRK[2], T. A. ALLEN[3], G. A. ELIAS[1];
[1]Dept. of Neurobio. and Behavior, UC Irvine, Irvine, CA; [2]Neurobio., Univ. of California San Diego, La Jolla, CA; [3]Psychology, Florida Intl. Univ., Miami, FL

Abstract: A critical feature of episodic memory is the ability to remember the order of events as they occurred during an experience. Accumulating evidence indicates that this capacity is supported by a network of structures centered on the hippocampus (HC) and prefrontal cortex (PFC). However, the distinct contributions of the HC and PFC remain unclear and very little is known about the specific role of other structures in this network. To address this issue, we trained rats to criterion on a nonspatial sequence memory task, which involves repeated presentations of a sequence of odors in a single odor port and requires animals to correctly identifying whether each odor is presented "in sequence" or "out of sequence." We then assessed task performance following localized temporary inactivations induced by infusions of fluorophore-conjugated muscimol, with saline infusions as control. Specifically, we examined the effects of inactivating HC or PFC (group 1) as well as regions with known anatomical and functional relationships with both structures, the perirhinal cortex and nucleus reuniens (PER, NRe; group 2). We found that overall performance was impaired after inactivation of each of these regions, indicating that they all make critical contributions to the ability to detect violations in the order of items presented in the sequence. Importantly, a detailed analysis of performance across odors and sequence positions revealed distinct patterns of impairment across regions. HC inactivations produced a modest impairment that was consistent across odors and positions, indicative of a general deficit in the ability to retrieve previously learned item-in-position associations. NRe inactivations produced an impairment of greater magnitude that was characterized by a flat accuracy gradient across all out of sequence items, suggesting an inability to take advantage of the representations that normally lead to higher performance on specific types of out of sequence items. In contrast, deficits following PFC inactivations appeared primarily due to an inability to keep track of the ordinal position in the sequence, and PER deficits to an inability to determine whether a repeated item is presented in its correct sequence position. Collectively these results demonstrate that each region makes distinct contributions to the memory of sequences of events.

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Poster

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NIDCD T32 DC010775 to G.A. Elias

Title: Prefrontal neurons track ordinal position within a sequence of events

Authors: *G. A. ELIAS[1], C.-W. NG[1], J. A. ASEM[1], T. A. ALLEN[2], N. J. FORTIN[1]; [1]Neurobio. & Behavior, Univ. of California, Irvine, Irvine, CA; [2]Psychology, Florida Intl. Univ., Miami, FL

Abstract: A key characteristic of episodic memory is its sequential organization. The hippocampus and prefrontal cortex have been identified as central structures within a network supporting the memory for sequences of events. In a recent study, we recorded neural activity in CA1 as rats performed a nonspatial odor sequence memory task and found that individual neurons coded for specific odors as well as specific conjunctions of odor and sequence position information (Allen et al., 2016, *JNeurosci*). Moreover, a significant proportion of CA1 neurons fired differentially whether odors were presented in the correct or incorrect sequence position, suggesting that the CA1 region plays a key role in sequence memory by supporting item-in-position associations. Here, we recorded prefrontal neurons (prelimbic region) in rats performing the same task to examine the role of the prefrontal cortex in this form of sequence memory. Briefly, rats received repeated presentations of a sequence of odors and had to determine whether each odor was presented in the correct sequence position or not. Preliminary results show that a majority of prefrontal cells (~65%) exhibited increases in activity in relation to specific task events and/or behavioral responses, and that this activity was modulated by ordinal position within the sequence. The majority of these cells (~60%) modulated their firing rate linearly; either increasing or decreasing their activity across sequence position. The remaining cells (~40%) exhibited a non-linear modulation across sequence positions, characterized either by preferential firing to the first and last sequence position (u-shaped function across positions) or to the intermediate positions (inverted-u function). Importantly, positional modulation in prefrontal neurons was present in time windows that preceded odor presentation, indicating that these cells represented the sequence position of the upcoming trial. This high prevalence and diversity of ordinal position coding in prefrontal neurons starkly contrasts with the high prevalence of odor and odor-in-position coding observed in CA1, highlighting that hippocampal and prefrontal

circuits make distinct contributions to sequence memory. Specifically, these results suggest that the prefrontal cortex plays a key role by dynamically tracking the animal's ordinal position within a sequence of events.

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Support: NIH Grant R01-NS075249

Title: Environmental enrichment normalizes hippocampal timing coding in a malformed hippocampus

Authors: *M. MAHONEY[1], A. E. HERNAN[1], W. CURRY[1], G. RICHARD[1], M. LUCAS[2], A. MASSEY[1], R. SCOTT[1,3];
[1]Univ. of Vermont, Burlington, VT; [2]Geisel Sch. of Med. at Dartmouth, Hanover, NH; [3]Univ. Col. London, London, United Kingdom

Abstract: Malformations of cortical development (MCDs) are structural and functional disruptions in neural networks. These network abnormalities are associated with learning and memory impairments and epilepsy. It is not known whether learning and memory can be improved, and if such improvement is possible, what the neural network therapeutic targets are. Injection of methylazoxymethanol acetate (MAM) at embryonic day (E) 17 produces a rat model that recapitulates many aspects of human MCDs, including cognitive deficits. We recently showed that environmental enrichment improves spatial cognition in MAM rats, although the mechanism for this is unknown. Using in vivo single unit recording, we show here that environmental enrichment completely resolves abnormal fine spike timing characteristics of CA1 hippocampal pyramidal cells in MAM animals. This is associated with improvement in spatial coherence, a surrogate measure of spatial cognition. We characterized fine spike timing using a neuronal endophenotype called a post-spike filter (PSF) and show that environmental enrichment restores the number of neurons that exhibit temporally modulated firing. Among neurons with temporal structure, the PSFs in MAM neurons show that neuronal firing is less precisely modulated, particularly in theta (6-12 Hz), indicating poorer temporal coding in MAM animals. Remarkably, environmental enrichment completely normalizes these changes. Moreover, PSF

parameters predict the spatial coherence of pyramidal cells, suggesting that coherence is dependent on circuit-level mechanisms that determine fine spike timing and that modulation of fine spike timing may be the mechanism by which spatial coherence is restored. MAM enriched animals have more PV interneurons than unenriched counterparts providing a potential cellular mechanism for improvements in temporal firing. This is the first system-level mechanism of cognitive improvement after environmental modification that has been shown in any model of human neurological disease. The results have far-reaching implications, as they suggest that even developmentally abnormal neural networks can be improved postnatally and that interneuron modulation could be a novel therapeutic approach for improving cognitive outcomes in neurological disease.

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Title: Access to a food entrainable oscillator improves performance on a daily time place learning task

Authors: ***C. M. THORPE**[1], K. WALL[2], L. M. LEWIS[2], A.-M. CHAULK[2], P. CHAULK[2], D. SKINNER[2];
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Abstract: In animals' environments many biologically significant events occur with spatial and temporal regularity. The ability of an animal to learn the spatiotemporal variability of stimuli is known as time-place learning (TPL). The present study investigated the interaction between a Food Entrainable Oscillator (FEO) and the Light Entrainable Oscillator (LEO) in a daily TPL task. Rats were trained in an operant conditioning chamber that contained two levers that distributed a food reward such that one lever provided a food reward in morning sessions, while the other lever provided food in afternoon sessions. We expected that having access to an FEO, in addition to the LEO, would provide rats with more accurate depictions of time of day, leading to better performance. Rats received either one meal per day (1M group), which allowed them to

have access to the FEO, or many meals per day (MM group), which did not allow them to have access to the FEO. As predicted, 1M rats had a significantly higher percentage of correct presses than MM rats across nine blocks. Once rats successfully learn the task, they will be made arrhythmic by shifting the light dark cycle by 3 hours per day as in Zelinski, Hong, & McDonald, 2014 to determine the effect of disruption of the LEO on the task. Manipulation of the light dark cycle will allow for a better understanding of the interaction between the LEO and FEO, therefore providing a more complete understanding of how circadian rhythms are related to learning and memory.

Disclosures: C.M. Thorpe: None. **K. Wall:** None. **L.M. Lewis:** None. **A. Chaulk:** None. **P. Chaulk:** None. **D. Skinner:** None.

Poster

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Title: Properties of cerebellar neurons during suprasecond time estimation.

Authors: *K. L. PARKER, B. J. DECORTE, Y. KIM, A. J. NESSLER, N. S. NARAYANAN; Dept. of Neurol., Univ. of Iowa, Iowa City, IA

Abstract: The cerebellum is involved in the temporal control of action. Here we explored the activity single neurons in the lateral (dentate) cerebellar nuclei (LCNs) in interval timing on the scale of seconds. We hypothesized that cerebellar neurons would similarly encode suprasecond epochs of time in both animals. We found that inactivating the cerebellum with GABA agonist, muscimol, impaired interval timing, while optogenetic stimulation and infusions of GABA antagonist, gabazine, could improve interval timing. This was particularly apparent in animals

with frontal dysfunction. To study LCNs neurons, we implanted recording electrodes in LCNs in rodents trained to estimate 3 and 12 second intervals. We found that LCNs have robust temporal processing and cue-evoked burst of low-frequency activity. To further explore this phenomenon, we analyze neuronal synchrony and population dynamics of neurons in the LCN during timing. We also examined LCN activity around movements and task events, and examined the relationship of cerebellar neuronal activity to cerebellar field potentials. We compared cerebellar LFP activity in rodents to EEG activity from cerebellar regions in humans. These data could have relevance for understanding the role of the cerebellum in temporal processing.

Disclosures: K.L. Parker: None. **B.J. DeCorte:** None. **Y. Kim:** None. **A.J. Nessler:** None. **N.S. Narayanan:** None.

Poster

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NARSAD Brain and Behavior Foundation

Leiber Investigators

Nellie Ball Research Trust Grant

Title: Timing impairments and cerebellar abnormalities in Prickle2 mice: implications for autism

Authors: *A. J. NESSLER[1], Y. KIM[1], S. WU[2], L. P. SOWERS[3], D. ANTIC[4], J. D. AXELROD[5], A. G. BASSUK[2], N. S. NARAYANAN[1], K. L. PARKER[1];
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Abstract: Autism spectrum disorders involve abnormalities across brain systems, resulting in a myriad of symptoms including behavior inflexibility, cognitive dysfunction, learning impairment, altered social interactions, and time perception. Recently, it was reported that a common variant in the Wnt gene *PRICKLE2* was present in individuals with autism. Providing additional support for the role of Prickle2 in autism, mice with disrupted Prickle2 exhibit similar phenotypes to individuals with autism including altered social interaction, learning impairments, and behavioral inflexibility. Additionally, Prickle2 disruption results in hippocampal neuronal

abnormalities including reduced dendritic branching, synapse number, and post-synaptic density size. Autism can involve the cerebellum. As Prickle2 is strongly expressed in Purkinje cells, this animal model presents a unique opportunity to investigate cerebellar abnormalities associated with autism-like phenotypes. We studied the performance of Prickle2 knockout mice in interval timing, a task that requires subjects to estimate an interval of several seconds using a motor response that involves the cerebellum. Prickle2 knockout mice trained in a fixed 12 second interval task were impaired during the first 3 days of learning. Over successive days of training they acquired accurate timing to the level of litter mate controls. Additionally, we explore structural abnormalities in animals with Prickle2 disruption using immunohistochemistry and stereology. These data suggest a role of the cerebellum in cognition and could inspire novel cerebellar-targeted treatments for cognitive impairments in autism spectrum disorders.

Disclosures: **A.J. Nessler:** None. **Y. Kim:** None. **S. Wu:** None. **L.P. Sowers:** None. **D. Antic:** None. **J.D. Axelrod:** None. **A.G. Bassuk:** None. **N.S. Narayanan:** None. **K.L. Parker:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Title: Initiation, continuation, adjustment, and termination: primary components of an ICAT model describing striatal-cerebellar interaction in temporal processing.

Authors: *E. PETTER, N. A. LUSK, W. H. MECK;
Duke Univ., Durham, NC

Abstract: The contributions of cortico-cerebellar and cortico-striatal circuits to timing and time perception has often been a point of contention. Dissociations between these two systems have been based on sub- vs. supra-second timing, duration-based vs. beat-based timing, and automatic vs. controlled timing. While some of these dichotomies arise out of different experimental constraints (e.g., psychophysical task or limitation in the range of time intervals studied), it is still uncertain whether these systems compete or cooperate with each other during temporal processing. We examined the cerebellum's contribution during timing tasks as fMRI suggest a role in temporal processing. This is supported as the neural architecture of the cerebellum makes it an ideal structure for temporal processing, with Purkinje cells in the cerebellar hemispheres integrating temporal aspects of salient stimuli. Furthermore electrophysiological studies show neural activity in cerebellar deep nuclei are modulated by the temporal regularity of stimulus presentations. In the current study, we attempted to identify the role of the cerebellum in both

sub- and supra-second timing using the duration bisection task with anchor durations of 200 vs. 800 ms and 2 vs. 8 s alternating within subjects and between sessions. To initially investigate the different components of the cerebellar-striatal network, we lesioned the dentate nucleus bilaterally. This caused impairments in reversal learning suggesting an inability to adjust. In order to further investigate components of temporal processing, we used in vivo recording techniques to measure temporal properties in striatal neurons during supra-second peak procedure tasks. This work in addition to previous literature suggests cortical-thalamo-striatal networks lend themselves to continuation of timing as well as initiation and adjustment during rhythmic stimuli. This data allows us to establish a parsimonious framework for the roles of the cerebellum and striatum in terms initiation, continuation, adjustment, and termination phases of temporal processing.

Disclosures: **E. Petter:** None. **N.A. Lusk:** None. **W.H. Meck:** None.

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Topic: H.01. Animal Cognition and Behavior

Title: A systematic exploration of temporal bisection models across sub- and supra-second ranges.

Authors: *N. A. LUSK, E. A. PETTER, W. H. MECK;
Psychology & Neurosci., Duke Univ., Durham, NC

Abstract: An integral component to the validity of timing models is their ability to accurately fit behavioral data from detection and discrimination tasks. Two of the most prominent timing models are the Sample Known Exactly (SKE), based on scalar timing theory, and the pseudo-logistic model (PLM). Currently, there is no standard by which behavioral data is fit, resulting in the implementation of both theoretical and atheoretical models, such as generalized logistic functions (L4P). Additionally, evidence accumulation models based on drift diffusion processes (DDM) have been utilized for modeling temporal bisection data. As many facets of timing behavior are quite nuanced, such as differences in precision across sub- and supra-sec durations, the variation in fitting models makes the interpretation of conflicting results difficult. Therefore, a comparative evaluation of these models was conducted to assess their ability to capture both quantitative and qualitative properties of timing data from the duration bisection procedure. As the DDM fits temporal bisection data in a fundamentally different way than the other three models, analysis was limited to within model comparisons using varying parameterizations.

Psychometric functions from rats, trained on a bisection task using sub-sec (200ms vs. 800ms.) and supra-sec (2s vs. 8s) conditions, were fit with each of the four models. Quantitative assessment of the fits used standard error of the regression (SE) as well as the Akaike Information Criterion (AIC) to evaluate model parsimony. The Weber fraction (WF) and point of subjective equality (PSE) were calculated for individuals and compared across both sub- and supra-sec durations within and between models. AIC analysis found the theoretical models vastly out performed the L4P, with the PLM being most parsimonious with the supra-sec data (AICc = -41.85), and the SKE and PLM both provided substantial explanatory power ($\Delta_i < 1$) for sub-sec data. Furthermore, differences existed in the PSE with the PLM and L4P being significantly different from the AM for sub-sec durations, while the PSE for the SKE was not. Three DDM models were analyzed with varying degrees of freedom. The DDM allowing for non-decision time, drift rate, and starting point to vary across signal durations was found to provide far great explanatory power ($\Delta_i > 10$) than the simpler models which only allowed for variation in drift rate or drift rate and starting point. These results demonstrate the need for a shift away from atheoretical models and towards theoretically based models such as the SKE or PLM, which provide greater parsimony as well as provide for greater qualitative analysis and interpretation of the data.

Disclosures: **N.A. Lusk:** None. **E.A. Petter:** None. **W.H. Meck:** None.

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Title: Geometric bisection by rats on the temporal bisection task is an artifact of delay discounting

Authors: *C. D. KOPEC[1], C. D. BRODY[2];
[1]Princeton Univ., Princeton, NJ; [2]HHMI / Princeton Univ., Princeton University, NJ

Abstract: The Temporal Bisection Task has been widely used for many decades in both human and nonhuman subjects to study the subjective perception of time. Briefly, on each trial subjects are presented with a probe duration and asked to report which of two previously learned reference durations the probe duration is closest to. The bisection point is given as the probe duration that elicits a “long” response 50% of the time. While human subjects tend to bisect near

the arithmetic mean of the two reference durations, nonhuman subjects tend to bisect near the geometric mean. One possible explanation for this difference is that human and nonhuman subjects either perceive the passage of time or compute decisions regarding temporal information differently. Here we present an alternative explanation.
Previously we developed a behavioral model that incorporates delay discounting of reward to guide behavior on a range of temporal processing tasks. This model not only provides a good fit to the rats' overall choice behavior, bisecting at the geometric mean, but also provides a good fit to the moment-by-moment response behavior during the performance of each trial and for a number of odd behaviors, including: multiple choice reversals during a single trial, choice reporting failures, and reversals in the psychometric function for durations shorter than the short reference duration or longer than the long reference duration. The model is also able to quantitatively predict the shift in the bisection point if the rewards associated with a "short" or "long" response are unequal.
We therefore hypothesized that if the temporal discounting of rewards is an important influence guiding response behavior, eliminating it as a motivating force should reveal the rat's true bisection point. To test this we modified the Temporal Bisection Task to eliminate any differences in discounted reward value between the two choice options at all moments in time. While rats bisected near the geometric mean on the classic Temporal Bisection Task, they shifted their bisection point to the arithmetic mean on the modified version of the task, in agreement with human performance. The difference in bisection point between human and nonhuman subjects is therefore likely not a result of any differences in how we process temporal information, but rather a result of different strategies being used to solve the same task. By eliminating discounted reward value as a decision-making strategy, leaving only the temporal information of the stimuli, we reveal that rats and humans perform similarly on the Temporal Bisection Task.

Disclosures: C.D. Kopec: None. **C.D. Brody:** None.

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Topic: H.01. Animal Cognition and Behavior

Title: Representations of elapsed time in hippocampal CA1 during a temporal bisection task

Authors: *A. SHIMBO[1,2], E.-I. IZAWA[1], S. FUJISAWA[2];
[1]Keio Univ., Tokyo, Japan; [2]Riken BSI, Wako, Japan

Abstract: Quantification of intervals is one of the most important ability for guiding appropriate behavior to survive. In particular, measuring temporal information in seconds to minutes range, referred to as interval timing, was involved in various behaviors, such as foraging, decision making, associative learning, and sequential motor learning. Although the importance of interval timing is recognized, the neural mechanisms of the interval timing are largely unknown. Recent studies have shown that hippocampal 'time cells' fire at temporarily successive moments in working memory task (Pastalkova et al., 2009; MacDonald et al., 2011), though the contributions of time cells to interval timing has not been investigated yet. To clarify the role of time cells in interval timing, we have newly developed a temporal bisection task. In this task, rats were required to discriminate 5 or 10 seconds interval, running on a treadmill, for making a correct choice. We recorded neural activities in CA1 during the periods in which rats were required to measure elapsed time from a start of running, and found that a subset of CA1 neurons showed firing activities depending on time from a trial start. These neurons fired as successive moment in the interval like time cells, and showed stable phase precessions on theta oscillations in CA1. These results indicate that time-dependent sequential activities of pyramidal cells in the hippocampus underlies the mechanisms for bisecting interval timing and may inform the passing time to other brain regions to solve the interval timing task.

Disclosures: **A. Shimbo:** None. **E. Izawa:** None. **S. Fujisawa:** None.

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Topic: H.01. Animal Cognition and Behavior

Title: Temporal and rate coding in hippocampus for integrations of non-spatial information

Authors: *S. TERADA;
Kyto Univ., Saitama, Japan

Abstract: Hippocampal pyramidal cells use both rate coding (i.e., place fields) and temporal coding (i.e., theta phase precession) for spatial navigation. However, much remains unclear how the hippocampus represents information and temporal relations during integrations of non-spatial events. Here we have newly developed a non-spatial associative memory task for rats under the head restriction, which is named cue-combination task. This task requires rats to associate two multimodal sensory cues (sound and odor cues) to determine a correct choice (left or right lever). Large-scale extracellular single unit recordings in the CA1 during this task revealed that some portion of the pyramidal cells were activated to specific sensory stimuli, combinations or

decisions, as the form of rate coding. On the other hand, the spiking of these neurons showed robust phase precession to theta oscillation, so that the past, current and future events were compressed and represented within a single cycle of theta oscillation. These results suggest that rate and temporal coding in hippocampal neurons independently contribute to integrations of multiple information for associative memory.

Disclosures: S. Terada: None.

Poster

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Title: A gap in time: Extending our knowledge of temporal processing deficit in the hiv-1 transgenic rat

Authors: *K. MCLAURIN, L. M. MORAN, H. LI, R. M. BOOZE, C. F. MACTUTUS; Dept. of Psychology, Univ. of South Carolina, Columbia, SC

Abstract: Approximately 50% of HIV-1 seropositive individuals develop HIV-1 associated neurocognitive disorders (HAND), which commonly include alterations in executive functions, such as inhibition, set shifting, and complex problem solving. Executive function deficits in HIV-1 are fairly well characterized, however, relatively few studies have explored the underlying dimensions of neurocognitive impairment in HIV-1. Deficits in temporal processing, caused by HIV-1, may manifest prior to early symptoms of impairment in higher level cognitive processes. Cross-modal prepulse inhibition (PPI), a translational measure of temporal processing, was studied in ovariectomized female HIV-1 transgenic (Tg) rats, which expresses 7 of the 9 HIV-1 genes constitutively throughout development. HIV-1 Tg animals exhibited an insensitivity to the manipulation of interstimulus interval (ISI) in both auditory PPI and visual PPI, replicating previously reported temporal processing deficits in HIV-1 Tg rats. To extend our knowledge of temporal processing deficits in the HIV-1 Tg rat, gap prepulse inhibition (gap-PPI) and gap threshold detection were also conducted. A relative insensitivity to the manipulation of ISI was observed in gap-PPI. HIV-1 Tg animals exhibited a profound differential sensitivity to the

manipulation of gap duration. Presence of the HIV-1 transgene was diagnosed with 88.9% accuracy using gap threshold detection. Understanding the generality of temporal processing deficits in the HIV-1 Tg rat is vital to modeling neurocognitive deficits observed in HAND and may aid in the development of a diagnostic screening tool.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: JSPS KAKENHI Grant 16K15196

Title: Neural processing for impulse control in medial prefrontal cortex and hippocampus

Authors: *A. MASUDA, C. SANO, S. FUJISAWA, S. ITOHARA;
RIKEN Brain Sci. Inst. - Wako, Wako-Shi, Saitama, Japan

Abstract: Impulsivity is a major characteristic of patients with drug abuse and psychological disorders. Dysfunctions of the medial prefrontal cortex (mPFC) and hippocampus can lead impulse control disorders, characterized by mistimed actions or decisions without due consideration of their consequences. However, it is not well known the relationships between activities of these brain regions. Here, we recorded the activities of single neurons in hippocampal CA1, dentate gyrus (DG), and mPFC, of mice performing a delay-based decision-making task in a T-maze. Large and small rewards were allocated to each side arm with or without a delay, respectively. The delay duration in the large-reward arm was progressively increased in each block (0, 5, 10, 20 and 40 s) in each session. The choice ratio to the large reward-delayed arm was negatively correlated with the delay durations. Putative excitatory and inhibitory neurons were identified by cell type classification with waveform parameters. Sizable amount of CA1 and DG excitatory neurons showed significant increases (about 40% of the population) in their firing rates during long (>20 s) delay periods. Increase of firing rates during long delay was found in mPFC neurons as well (about 40% of the population). Next, we investigated the place-dependency of the delay-activated neurons by switching the large reward-delayed arm with the small reward-non-delayed arm. Numerous (about 70 %) delay-activated neurons found in CA1 and DG were location selective (neurons specifically fired on one side) whereas few delay-activated neurons in mPFC (30 %) were location selective. Our findings

indicate that mPFC neurons encode the information for discounting reward values by a delay and that hippocampal neurons encode the information for both value and positions.

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Title: Hippocampal and prefrontal cortical temporal coding in a model of cortical malformation

Authors: ***A. E. HERNAN**[1], M. MAHONEY[1], R. C. SCOTT[1,2];
[1]Neurolog. Sci., Univ. of Vermont Col. of Med., Burlington, VT; [2]Inst. of Child Hlth., Univ. Col. London, London, United Kingdom

Abstract: Malformations of cortical development (MCDs) are the result of an insult during the neuronal migration process that leads to structural and functional abnormalities in neural networks. In humans, MCDs are often associated with intractable epilepsy and cognitive deficits. In this study we used the embryonic day 17 methylazoxymethanol acetate (MAM) model to examine the impact of these MCDs, independently of seizures, on neural networks that subserve cognition. Using concurrent single unit recording in the medial prefrontal cortex (mPFC) and CA1 of the hippocampus during a working memory task that requires cross-talk between the two structures, we hypothesized that MAM animals would have significant alterations in rate and temporal coding within and between CA1 and mPFC that underlie cognitive deficits. To address this hypothesis, we used a generalized linear modeling (GLM) approach to directly model neuronal firing in both brain regions in MAM and control animals at baseline and during a delayed non-match to sample task. Preliminary data indicate that this GLM approach robustly captures *in vivo* spike train dynamics, that neuronal firing is modulated by several relevant task parameters and that significant firing correlations exist between neurons in CA1 and mPFC. We find that hippocampal temporal and rate coding are disrupted in MAM animals relative to controls; however the relationships between this and abnormalities in PFC, and the relationships between these parameters and cognition are currently being investigated. Understanding the systems-level mechanism of cognitive deficits in animals with MCDs could lead to novel therapeutic targets that maximize cognition.

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K08 NS078100

Title: Optogenetic manipulation of frontostriatal circuits during interval timing

Authors: ***E. EMMONS**, B. DECORTE, K. PARKER, N. NARAYANAN;
Univ. of Iowa, Iowa City, IA

Abstract: Here we investigated the dynamics of temporal processing in the medial frontal cortex (MFC) and striatum in an interval-timing task. This elementary cognitive task involves working memory and attention and, importantly, is impaired in schizophrenia. First, we trained rats on an interval-timing task with a 12-second delay. Following training, animals were injected in the MFC with viral vectors to express ChR2 and ArchT optogenetic proteins. Animals were simultaneously implanted with microwire electrode arrays surrounding a fiberoptic cannula in the dorsomedial striatum. After waiting 4 weeks for viral expression, we inhibited and stimulated neurons in the dorsomedial striatum with 561-nm and 473-nm light, respectively. Medial frontal projections to the striatum that expressed optogenetic proteins were directly modulated by laser administration. Interval-timing behavior was altered in response to ArchT stimulation and subsequent inhibition of frontostriatal projections during the trial. Behavior was also altered in response to ChR2 stimulation at 4 and 20 Hz. Electrophysiological activity recorded at the site of stimulation in the dorsomedial striatum demonstrated a pronounced change in neural activity during both ChR2 and ArchT stimulation. Gradual changes in neural activity over time, or "ramping" activity, were found in striatal neurons during interval-timing behavior. These patterns of "ramping" activity are one possibility for the encoding of temporal information at a neural level. Ramping was diminished in the striatum when interval timing was impaired, suggesting that temporal information may be represented at the neural level by ramping activity. We also examined the population dynamics of these effects. These data demonstrate the importance of frontostriatal circuits in basic cognitive processing and suggest strategies for further elucidation of cognition.

Disclosures: **E. Emmons:** None. **B. DeCorte:** None. **K. Parker:** None. **N. Narayanan:** None.

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Title: Direct and indirect pathway function in interval timing behavior

Authors: ***B. J. DECORTE**[1,2], M. MATELL[3], N. NARAYANAN[2];
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Abstract: Interval timing, the perception of time in the seconds-to-minutes range, is critical for adaptive behavior and is impaired in a variety of illnesses (e.g., Parkinson's disease, Huntington's disease, Autism Spectrum Disorder, etc.). Interval timing is often examined using the peak interval (PI) procedure. In a typical PI task, the onset of a discriminative stimulus (e.g., houselight) indicates that reinforcement may be earned for responding after a set "criterion duration" has elapsed (e.g., 6 seconds). During non-reinforced probe trials, in which the stimulus is presented for 3-4 times the length of the criterion duration, average response rates resemble a Gaussian distribution, peaking around this criterion time. In the current study, we examined how the direct and indirect pathways mediate responding during the PI procedure. Rats were trained on a PI task in which a houselight signaled a 6 second delay to reward. Once trained, cannulas were implanted into the dorsomedial striatum. We infused the D1 antagonist (SCH-23390) or the D2 antagonist (Sulpride) to manipulate direct and indirect pathway function, respectively. We found that the D2 antagonist shifted responding rightward, consistent with prior work on systemic injections of D2 antagonists and D2-over expressing mice. These results suggest striatal D2 receptor blockade slowed internal clock speed, and provide insight into the role of dorsomedial striatum and temporal control of action.

Disclosures: **B.J. Decorte:** None. **M. Matell:** None. **N. Narayanan:** None.

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Title: Hallucinogen-induced alteration of timing performance is mediated by 5-HT2A: Insights from a mouse discrete-trials interval timing task

Authors: *L. M. KLEIN[1], M. A. GEYER[2], A. L. HALBERSTADT[2];
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Abstract: Interval timing, i.e. discrimination of durations in the seconds to minutes range, is imperative for organization of behavioral sequences, anticipation of outcomes, and information processing. Altered interval timing disrupts assessment of relationships between sensory stimuli, resulting in disorganized or contextually inappropriate behavior. Schizophrenia (SZ) patients demonstrate deficits in temporal judgment that may underlie positive symptoms such as delusions and misattribution of causality. Classical hallucinogens effectively model the positive symptoms of SZ across species and reliably compromise the accuracy and precision of temporal discrimination in humans. Similar mechanisms may thus underlie timing alterations in SZ and hallucinogen intoxication. Though many perceptual effects of hallucinogens are associated with 5-HT2A receptor activation, the mechanism by which hallucinogens alter timing is unclear. We developed a discrete-trials interval timing task for mice, optimized to assess interval timing during pharmacological manipulation. C57BL/6J mice were presented with two levers following a variable interval after trial initiation (10 intervals; 2.5-10.5 s). Responding on lever A was reinforced if the interval was <6.5 s, and responding on lever B was reinforced if the interval was >6.5 s. Proportional choice for lever B at each test duration was analyzed using a 2-parameter logistic function, allowing estimation of the bisection point (T50), a measure of clock speed, and the difference limen (DL), a measure of timing precision. Challenge with the structurally distinct hallucinogens lysergic acid diethylamide (LSD), psilocin, and 2,5-dimethoxy-4-iodoamphetamine (DOI) during this task revealed consistent dose-dependent alterations in timing across the 3 structural classes: reduced % lever B responding at long intervals and increased T50 (LSD: 1 mg/kg IP; psilocin: 0.8 mg/kg SC; DOI: 3 mg/kg IP), and increased DL (psilocin: 0.8 mg/kg SC; DOI: 3 mg/kg IP). Administration of 25CN-NBOH, an agonist selective for 5-HT2A, dose-dependently recapitulated all three alterations of timing (6 mg/kg SC), suggesting that hallucinogens alter timing via their effects on 5-HT2A. The effects of DOI (3 mg/kg IP) on timing were blocked by the highly selective 5-HT2A antagonist M100907 (0.03 mg/kg SC), but

were unaffected by the 5-HT2C selective antagonist SB242084, consistent with a 5-HT2A-dependent mechanism. These findings further validate the translational utility of the mouse discrete-trials interval timing task and clarify the mechanism of a key perceptual alteration induced by hallucinogens with relevance to SZ.

Disclosures: **L.M. Klein:** None. **M.A. Geyer:** A. Employment/Salary (full or part-time): University of California, San Diego. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; NIDA, NIMH, U.S. Veteran's Administration VISN 22 Mental Illness Research, Education, and Clinical Center, Intracellular Therapeutics, Johnson & Johnson. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); San Diego Instruments. F. Consulting Fees (e.g., advisory boards); Amgen, Abbott, Cerca, Dart Neuroscience, Merck, Omeros, Otsuka, Sunovion, Lundbeck, Neurocrine, Takeda, Teva. **A.L. Halberstadt:** A. Employment/Salary (full or part-time): University of California, San Diego. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; NIMH, Brain & Behavior Research Foundation.

Poster

643. Neural Circuits for Timing, Temporal Processing, and Sequences

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 643.23/KKK69

Topic: H.01. Animal Cognition and Behavior

Title: Sodium butyrate facilitates the acquisition of the "Stop" response threshold for peak-interval timing - Role of Histone acetylation in temporal extinction learning.

Authors: *B. A. CAHN, W. H. MECK, C. L. WILLIAMS;
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Abstract: In the peak-interval (PI) timing procedure, the subject is first presented with fixed-interval (FI) trials only, during which the onset of a light or tone reliably predicts the time at which reinforcement will be made available (target duration). In order to characterize the accuracy and precision of interval timing, un-reinforced probe trials are randomly intermixed within an experimental session after sufficient training with FI trials. For a probe trial, the signal stays on long past the target duration and no reinforcement is provided. Only by maintaining partial reinforcement (e.g., continued reinforcement on FI trials and non-reinforcement on probe

trials) across multiple sessions do subjects learn to stop responding once the target duration has passed. Despite these observations, there is still little understanding concerning how the "Start" and "Stop" response thresholds are acquired and what the underlying molecular mechanisms for the partial-extinction learning on un-reinforced probe trials might be. A pivotal role has been demonstrated for histone acetyltransferase CREB-binding protein and histone deacetylases (HDACs) in the epigenetic modification of memory and synaptic plasticity. HDAC inhibitors block the activity of histone deacetylases and thus increase histone acetylation thereby facilitating various types of learning and memory. As a consequence of these findings, the current experiment was designed to investigate whether systemic injection of the potent histone deacetylase inhibitor sodium butyrate (NaB) would facilitate the acquisition of the "Stop" response threshold in a 20-s PI procedure. Following 14 sessions of baseline FI 20-s training, rats were transferred to a 20-s PI procedure and given ip injections of either 1.2 g/kg NaB in a volume of 0.5 mL (n=12) or saline (n=12) for 5 consecutive sessions, after which the rats receive another 5 sessions of PI testing with no injections. The results indicated a significant effect on the development of temporal inhibition following the target duration as early as the first 2 hr session. This improvement in temporal sensitivity as measured by the curvature of the PI response functions continued through the "wash-out/recovery" phase until the final 2 sessions at which time both the NaB and control rats demonstrated nearly symmetrical peak functions centered around 20 s. Follow-up microinjection studies will determine the role of histone deacetylases in the dorsal and ventral striatum in setting the "Start" and "Stop" thresholds for interval timing.

Disclosures: B.A. Cahn: None. **W.H. Meck:** None. **C.L. Williams:** None.

Poster

643. Neural Circuits for Timing, Temporal Processing, and Sequences

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Topic: H.01. Animal Cognition and Behavior

Support: MH065561

MH73057

Title: Prelimbic cortex activation correlates with SSRI fluoxetine's effectiveness to reduce the impact of emotional distracters on interval timing

Authors: *A. R. MATTHEWS[1], M. BUHUSI[2], C. V. BUHUSI[2];
[1]Dept. of Psychology, [2]Utah State Univ., Logan, UT

Abstract: Selective serotonin reuptake inhibitors (SSRI) are a primary medication for affective disorders like depression and anxiety. SSRI fluoxetine (FLX) has beneficial effects on attention and working memory, e.g., decreasing emotional response to negative events. We hypothesized that FLX improves maintenance of information in working memory, and decreases the disruptive effect of emotional events on cognitive processing. Among the processes impaired by emotional events, and whose dysregulation is documented in affective disorders, is timekeeping in the seconds-to-minutes range (interval timing). Presentation of task-irrelevant emotional distracters during a peak-interval (PI) procedure results in considerable delays in responding relative to neutral distracters (Matthews et al 2012 Front Int Neurosci 6: 111). Here we examined the neural correlates of chronically administered FLX in regard to the delaying effect of anxiety-inducing distracters on interval timing. Rats were trained in a PI procedure as in Mathews et al. 2012. Half the rats received 16 days of chronic systemic FLX administration, while the others received vehicle injections (VEH). Afterwards, rats were tested by presenting an anxiety-inducing distracter and evaluating the timing delay in distracter trials relative to no-distracter (PI) trials. Chronic FLX administration significantly decreased the delaying-effect of the anxiety-inducing distracter relative to VEH. The timing delay after the anxiety-inducing distracter (relative to PI trials) was inversely correlated with neuronal activation (cFos counts) in prelimbic cortex (PrL) (R^2=0.47, $p<0.01$), but not in infralimbic cortex (R^2=0.14, $p>0.05$): The higher the PrL activation the more effective was FLX in reducing the timing delay after the anxiety-inducing distracter, suggesting that one locus of action of FLX in reducing the delay after anxiety-inducing distracters is PrL. Indeed, the delay after anxiety-inducing distracters was reduced by local infusions of FLX into PrL (Matthews et al. Program No. 85.18, 2015 Neuroscience Meeting Planner). Together, these results identify PrL as a structure involved in the relative sharing of resources allocated to interval timing and emotion (Buhusi & Meck 2009 Philos Trans R Soc B 364: 1875-85).

Disclosures: **A.R. Matthews:** None. **M. Buhusi:** None. **C.V. Buhusi:** None.

Poster

644. Human Cognition and Memory IV

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Program#/Poster#: 644.01/LLL1

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01-MH062500

Title: The impact of vmPFC and hippocampal lesions on episodic segmentation and relational memory

Authors: *M. R. DULAS[1], M. C. DUFF[2], N. J. COHEN[1];
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Abstract: Previous work in temporal order memory has shown that, in healthy participants, context boundaries result in segmentation effects, wherein within-context information is remembered as closer together than across-context information, even when equidistant. Further, imaging evidence has suggested the ventromedial prefrontal cortex (vmPFC) and the hippocampus play critical roles in these relational memory and segmenting processes. The present study investigated segmentation effects for spatial information, their interaction with relational memory, as well as the impact of lesions to the vmPFC and hippocampus on these effects. On each block of the task, participants studied 8 objects, presented one at a time on different locations of a grid, divided across two contexts (scenes). At test, participants judged how far apart pairs of these objects were, and also answered two relational memory questions: in which context and in which half of the set of 8 (first or second) did each object appear. Participants included both patients with hippocampal and vmPFC lesions, as well as healthy comparison individuals. Healthy participants showed robust segmentation effects, rating within-context pairs as closer together than equidistant across-context pairs. Further, these segmentation effects were positively correlated with relational memory accuracy. Hippocampal patients were at chance for these judgments, regardless of the distance, and also showed significant impairments for both relational memory questions. Patients with vmPFC lesions performed similarly to healthy comparisons on all tasks, except they did not show segmentation effects, rating equidistant across-context and within-context pairs as the same distance. The results suggest that, in healthy adults, episodic segmentation may be useful in supporting later relational memory judgments. Further, while the hippocampus is likely necessary for all forms of spatial and relational memory judgments, even for small sets of items, the vmPFC may play a particular role in using contextual boundaries to bias memory representations via episodic segmentation.

Disclosures: **M.R. Dulas:** None. **M.C. Duff:** None. **N.J. Cohen:** None.

Poster

644. Human Cognition and Memory IV

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Topic: H.02. Human Cognition and Behavior

Support: Early Researcher Award from the Ontario Government (to M.D.B.)

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Title: Enhanced temporal order memory for events preceding contextual boundaries during navigation

Authors: *I. BRUNEC[1,2], J. D. OZUBKO[3], M. MOSCOVITCH[1,2], M. D. BARENSE[1,2]; [1]Psychology, Univ. of Toronto, Toronto, ON, Canada; [2]Rotman Res. Institute, Baycrest, Toronto, ON, Canada; [3]Dept. of Psychology, SUNY Geneseo, Geneseo, NY

Abstract: Route representations learned during spatial navigation are reconstructed from memory by relying on turns as boundaries segmenting the path. The ability to detect and predict contextual boundaries has been shown to depend on the hippocampus (Zacks et al., 2007). In rodents, spatial boundaries are associated with hippocampal cell ensemble preplay, which is thought to reflect the prediction of turns and route trajectory (Erdem & Hasselmo, 2012). Here, we aimed to join the literatures on event boundaries and navigation. We used a naturalistic virtual reality navigation paradigm to test memory for spatial and temporal contextual information for events preceding event boundaries. Half of the participants actively navigated along a route and half were passively led through the route. Participants in both conditions were stopped for different durations at several intersections which were either followed by turns (event boundaries) or stretches of road in same direction (no boundary). We tested participants' ability to discriminate between the ordinal positions and wait durations for pairs of intersections along the route. We found that memory for temporal order, but not event duration, was significantly improved for events followed by boundaries. This effect was unique to the active navigation condition, suggesting that passive route following does not result in a similar improvement in memory, despite the fact that intersections followed by turns were subjectively recollected more in both conditions. We propose that the observed memory boost is advantageous in predicting and segmenting upcoming events along a route and serves as a mechanism of encoding navigationally salient events. Such enhanced memory further enables more successful separation of the sequential positions in which spatial locations were encountered. These findings are consistent with the notion that recollection supports whichever feature is most relevant to the task at hand, in line with the hippocampus supporting consciously processed information.

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Poster

644. Human Cognition and Memory IV

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Topic: H.02. Human Cognition and Behavior

Support: DFG: BU 2670/2-1

Title: Striatal novelty responses reverse with retrieval demands

Authors: *N. A. HERWEG[1], T. SOMMER[1], N. BUNZECK[1,2];
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Abstract: The striatum and pallidum have been implicated in relaying novelty signals from the hippocampus to dopaminergic midbrain neurons. The same areas, however, also respond to retrieval success in recognition tasks, suggesting that these areas do not code novelty per se but dynamically adapt to the current behavioral context. Here, we used fMRI in healthy humans to test the prediction that encoding vs. retrieval orientation, as induced by task context, modulates striatal old/new effects. To this end, participants were initially familiarized with a set of scene stimuli in a target detection task. Subsequently, they viewed these old images intermixed with new ones and either classified them (i) as indoor vs. outdoor (encoding) or (ii) as old vs. new (retrieval), while fMRI and eye tracking data were recorded simultaneously. After leaving the scanner, subjects performed a final recognition task. As hypothesized, a cluster including parts of the striatum and pallidum displayed a significant interaction of task context and old/new status, which was driven by stronger responses to novel images in the encoding context and the reversed pattern, i.e. stronger responses to old images, in the retrieval context. An interaction was also evident in pupil size. Here, the old>new effect was more pronounced during retrieval than during encoding mode. Taken together, our findings provide compelling evidence that mnemonic signals in the basal ganglia flexibly adapt to explicit recognition demands. This mechanism might be mediated by dopaminergic neuromodulation and indicates that the degree of salience of old and new information is context dependent.

Disclosures: **N.A. Herweg:** None. **T. Sommer:** None. **N. Bunzeck:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

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JSPS Postdoctoral Fellowships for Research Abroad

Title: Spatio-temporal dynamics of short-term working memory maintenance and scanning of verbal information

Authors: *T. KAMBARA[1,2], E. BROWN[3], J.-W. JEONG[1,4], N. OFEN[1,5], Y. NAKAI[1], E. ASANO[1,4];
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Abstract: Humans briefly maintain speech sounds such as those conveyed in verbal phrases and scan those mental representations to generate relevant responses during verbal communication. Here, we determined the spatio-temporal dynamics of such short-term maintenance and scanning of verbal information, by intracranially measuring high-gamma activity at 70-110 Hz during a working-memory task. Patients listened to 2 or 4 letters and were instructed to remember those letters over a two-second interval, following which they were asked to determine if a subsequent target letter was presented earlier in that trial. The auditory presentation of the letters sequentially elicited high-gamma augmentation in the superior-temporal and precentral gyri, bilaterally. During the two-second maintenance period, high-gamma augmentation was observed in the precentral gyri with left hemispheric dominance, and this occurred to a greater extent during the maintenance of 4 compared to 2 letters. During the scanning period following target presentation, high-gamma augmentation was observed in the inferior-frontal and supra-marginal gyri also with left hemispheric dominance. Finally, at the onset of the response, high-gamma augmentation was observed in the pre- and post-central gyri, bilaterally. These results support the notion that short-term maintenance of verbal information is supported by the precentral gyrus, whereas scanning to determine of a match for an auditory stimulus is supported particularly by the left inferior-frontal and supra-marginal gyri.

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Poster

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Title: Evidence for persistent activity-based attractor states from human single-neurons during short term memory maintenance

Authors: *J. KAMINSKI[1], S. SULLIVAN[1], J. CHUNG[2], I. ROSS[3], A. MAMELAK[1], U. RUTISHAUSER[1,2,4];
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Abstract: Sustained neuronal firing is thought to be a principle mechanism by which information is maintained in short term memory (STM). Such activity has been hypothesized to form attractor states, which are mathematical representations of preferred and stable network configurations. This hypothesis rests on theoretical models and experimental evidence in macaques but has not been demonstrated in humans. We performed human single-neuron recordings in neurosurgical patients to test for the presence and properties of attractors during the maintenance of information in STM. We recorded from two regions: the medial temporal lobe and the medial frontal cortex. We used a modified Sternberg task with pictures as study material. To improve neuronal responses, images were selected for each patient individually using a screening task. We observed 83 (12.3%) neurons in the screening task, mainly in hippocampus and amygdala, with selectivity for one of the images. These cells showed sustained activity that was selective for the content of STM during the delay period of the task. We analyzed the dynamics of neuronal activity using demixed PCA (dPCA) in order to extract components relevant for encoding picture identity. An analysis of neural trajectories in dPCA space during the time when subjects held an item in memory showed that the multidimensional distance between conditions with different images held in memory was significantly larger than expected by chance. At the same time, we also observed that the velocity in state space was low and not significantly different from baseline levels. Together, this indicates neural trajectories that form stable attractors. Furthermore, we found that the quality of the identified attractors was relevant for STM performance because the extent of drift away from attractors in each trial predicted later retrieval accuracy and speed of response. This observation is the first evidence in humans that

single-neurons code information in STM by means of persistent firing activity, and that this persistent activity forms stable attractors.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: CERC 215063

Title: Targeted training: Assessing the effects of online brain training on cognitive function

Authors: ***K. M. LYONS**[1,2], A. PEARCE[2,1], T. NGUYEN[2,1], A. M. OWEN[2,1], B. STOJANOSKI[2,1];
[1]Univ. of Western Ontario, London, ON, Canada; [2]Brain and Mind Inst., London, ON, Canada

Abstract: 'Brain training' is built on the idea that general cognitive functioning can be improved through the regular use of online computerized tasks that target specific cognitive domains. While the idea that brain training will improve general cognitive functioning is appealing, reliable evidence in support of this is unconvincing (Thompson et al., 2013; Owen et al., 2010). What remains contentious is not that performance cannot be improved by training, but, instead, whether training transfers to unrelated tasks. Using Cambridge Brain Sciences, a set of online cognitive assessment tests, the current study investigated whether extensive training on a working memory task produced improved performance on two tasks with similar or different neural and cognitive profiles. Participants first benchmarked on the two test tasks, spatial span and digit span, which served as their baseline measure. They then trained on a visuospatial working memory task, for thirty minutes a day, five days a week for four weeks (ten hours total). One day after training was complete, participants were tested again on the spatial span and digit span tests. As expected, performance on the training task (visuospatial working memory) significantly improved over the training period ($p=0.016$), and this provided evidence for direct transfer. However, when performance on the test tasks was compared before and after training, no change was found in mean score for either the spatial span ($p=0.26$) or the digit span tasks ($p=0.80$). Perhaps only those who benefited the most from training showed transferrable gains on the test tasks? To test this hypothesis, "high trainers" were selected based on their rate (slope) of improvement on visuospatial working memory task. Despite benefiting more from training, this

did not transfer to improved performance on the spatial or digit span task. Our results suggest that participants show direct transfer (to the same task) only after extensive training and, even then, no matter how beneficial the training, no measurable transfer to any other task is evident.

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Poster

644. Human Cognition and Memory IV

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Foundation Adelis

MH-055687 to Michael Kahana

Title: Evoked potential to word presentation correlates with its inherent recall probability

Authors: *M. KATKOV[1], M. TSODYKS[1,2];
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Abstract: *In this contribution we provide electrophysiological evidence for recently proposed model of memory retrieval from long-term memory, according to which the size of encoding representation of a given word is positively correlated with the probability to recall this word in a free recall experiment.*
Despite the ability of humans to memorize a huge amount of information; recalling this information without relevant cues is a challenging task. This is manifested in, for example, free recall experiments, where participants are prompted to recall as many words as possible from just presented list of randomly assembled words. Participants are making mistakes even for shortest lists of 2-5 items with progressively smaller fraction of correctly recalled words with increasingly longer lists. We have recently proposed a mechanism for memory retrieval which attributes limited recall capacity to two basic phenomena. 1) Items in long-term memory network are represented by random groups of neurons. 2) In the absence of external cues, just recalled item (JRI) plays a role of an internal cue, triggering a recall of an item having the largest overlap with JRI. This mechanism inherently leads to the existence of “easy” and “difficult” words for recall - statistically items having larger representation are easier to recall since their overlaps with other representations will be larger on average. Analysis of behavioral data revealed that

distribution of recall difficulties across different words is indeed consistent for different randomly selected groups of participants. In the current contribution we analysed scalp electrical activity in the brain (EEG) collected in the M. Kahana lab (UPenn) during list acquisition. In particular, we computed the average (across all presentations for all participants) normalized evoked response for each word separately. Next, we computed correlation coefficient of normalized responses at each time point and each electrode with the fraction of times the word was recalled when presented. Results showed two waves of highly correlated activity at 250-400ms and 450-700ms with the largest correlation coefficient in a single electrode being 0.25 for the first wave and 0.15 for the second wave. This result is consistent with our basic assumption that words characterized by a larger group of encoding neurons (and hence the ones that reactivate larger neuronal population during presentation) have intrinsically higher probability to be recalled.

Disclosures: **M. Katkov:** None. **M. Tsodyks:** None.

Poster

644. Human Cognition and Memory IV

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Topic: H.02. Human Cognition and Behavior

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Title: Brain regions associated with value-driven encoding of novel kaleidoscope images

Authors: ***M. S. COHEN**, L. Y. CHENG, K. HAQUE, K. A. PALLER, P. J. REBER; Dept. of Psychology, Northwestern Univ., Evanston, IL

Abstract: Information tends to be remembered better when it is known to have relatively high value for the future. This effect is thought to occur either due to dopamine-driven strengthening of encoding in the hippocampus (e.g., Adcock et al., 2006), or due to modulation of the use of semantic strategies to encode words (e.g., Cohen et al., 2016). In the present study, we examined whether value can also strengthen memory for abstract, novel kaleidoscope images, for which little semantic processing can be done. Each image was presented in one of four quadrants, with two images, one high-value item and one low-value item, presented in different quadrants on each encoding trial. At test, individual items were presented centrally, and participants attempted to judge whether a given item was old or new, as well as recall the original spatial location.

Multiple study-test cycles were used, with aggregate feedback provided after each test, in order to encourage participants to use metacognition to develop strategies that maximize their point score. After all cycles were complete, a second test, a forced-choice recognition test with highly similar foils, was completed. Accuracy on the yes-no quadrant test was higher for high-value items, suggesting that encoding was modulated by value in this paradigm. Results from fMRI showed that successful encoding of kaleidoscope images and spatial source information (quadrant at study) was associated with increased activity in a network of brain regions including superior and inferior lateral occipital cortex, posterior parietal, inferior lateral temporal/fusiform cortex, and putamen, relative to trials in which neither image was successfully encoded. There were also related regions in which activity was stronger when only a high-value image was successfully encoded, relative to trials in which only a low-value image was encoded successfully. Thus, we can begin to understand the neural circuitry in which modulation of activity by value leads to strengthening of subsequent memory for both the item and its spatial location. Behavioral pilot results with this paradigm also indicated that while high-value items showed enhanced recognition driven by explicit memory, the accuracy of guess responses on the forced-choice test, believed to be driven by implicit memory, tended to be greater for low-value than for high-value items. Thus, our results also provide a first step towards understanding the encoding that impacts the relative weighting of information from implicit and explicit memory systems in memory judgments.

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Poster

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Topic: H.02. Human Cognition and Behavior

Title: Familiarity-novelty detection is an intrinsic property of cortical microcircuits with NMDA receptor-dependent synaptic plasticity

Authors: *X. ZHANG[1], H. JU[2], T. B. PENNEY[3], A. M. J. VANDONGEN[2];
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Abstract: Familiarity and recollection are two processes underlying recognition memory. Evidence from functional imaging studies shows that cortical regions surrounding the

hippocampus become active when a human subject senses a familiar input, whereas recollection relies on activation of the hippocampus itself. To deepen our understanding of familiarity-novelty detection, we simulated biologically plausible neural network models of cortical microcircuits consisting of 2,000 – 15,000 spiking neurons with random recurrent connections, following the "liquid state machine" paradigm. Network activity evoked by the sensory input (spatially-differentiated images) altered synaptic efficacy through NMDA receptor-dependent synaptic plasticity, which in turn resulted in the network responding more strongly to a previously seen image via unsupervised learning. Small networks of 2,000 spiking neurons were able to encode the features of a human face image and identifiy it accurately out of a set of 30 test images. Large networks of 15,000 spiking neurons were able to identify multiple familiar faces simultaneously with high accuracy. When the simulated network model became sufficiently complicated in structure, multiple familiarity traces could be retained in the same network by forming partially-overlapping subnetworks. These subnetworks were composed of potentiated and depressed synapses that were modulated by the corresponding sensory inputs. A very large number of input-representative subnetworks that differ slightly from each other can co-exist in the network, giving the model a promisingly high capacity. Therefore, we suggest that cortical microcircuits with NMDA receptor-dependent synaptic plasticity have an intrinsic ability for detecting familiar sensory input patterns. This ability does not require a specialized wiring diagram or supervision, and can be expected to emerge naturally in developing cortical circuits. Meanwhile, electrophysiological recordings from animals indicate that single cell activity within the cortical regions can represent familiarity. Therefore in addition to network firing rate response, we also investigated neuronal activity before and after sensory exposure and found that "grandmother-cell" neurons exist in our network model. Fisher's discriminant analysis was applied to select the critical neurons whose activity can be used to predict input patterns. Intriguingly, as sensory exposure was prolonged, the selected critical neurons tended to appear more at deeper layers of the network model, which might suggest a strategy of recruiting hidden circuits in the network for incremental information storage.

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Poster

644. Human Cognition and Memory IV

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 644.10/LLL10

Topic: H.02. Human Cognition and Behavior

Support: Fredrik O Ingrid Thuring 2015-00153

Title: Time-resolved single-trial multivariate pattern decoding as a continuous measure of working memory load using EEG spectral power

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Abstract: Decoding cognitive information from brain signals by using multivariate pattern classification methods is becoming increasingly popular. Most studies have however only classified the information into pre-defined states which consequently limits the possible applications. Working Memory (WM), the capacity to remember and manipulate relevant information during a short time period, is central for human cognition. Because many psychiatric populations (i.e. ADHD, depression, substance abuse and many more) have impaired WM capacity, the potentials of extracting WM load and applying it as a feedback signal in a training protocol to enhance the underlying brain activity pattern, is supported by observations that individuals with high WM capacity evoke a more discriminative activity pattern when performing WM tasks. This study investigates the temporal dynamics of WM-induced EEG spectral power patterns and the feasibility of extracting a continuous measure of WM load by using time-resolved single-trial multivariate pattern decoding.
Brain activity was recorded, using a 64-active EEG-channel system, on 16 healthy individuals while they performed two versions of the n-back task. Specifically, the 1- and 2-back tasks were performed in two separate blocs, during which participants were asked to respond with a button press, yes or no, for each stimulus, whether it matched the stimulus presented 1 or 2 steps back (1-back and 2-back task, respectively). A complex EEG power spectrum over the scalp is observed as WM load increases, including a significant decrease in alpha power (~8 to 12Hz) over parietal electrode locations and significant increase in theta power (~3 to 7Hz) over frontal electrode locations. Moreover, temporal dynamic changes in the EEG power spectrum are observed at the presentation of a visual stimulus for theta but not for alpha power suggesting that different underlying mechanisms of WM are reflected by these oscillations. By applying multivariate pattern decoding, using linear regularized regression, the output of the binary classifier is shown to correlate with the strength of the EEG activity pattern that is induced as WM load increases, as well as with overt behavior, suggesting its applicability as a continuous measure in online monitoring and as a feedback signal in rehabilitation protocols. These results shed new light on the extraction of cognitive information from EEG spectral power by using time-resolved single-trial multivariate pattern decoding.

Disclosures: **E. Astrand:** None.

Poster

644. Human Cognition and Memory IV

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 644.11/LLL11

Topic: H.02. Human Cognition and Behavior

Support: CIHR

Title: Modulation of pupillary light responses by saccade preparation, working memory, and microstimulation of the superior colliculus

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Abstract: Pupil size changes constantly, mainly to regulate the amount of light entering to the retina, with pupil constriction to luminance increases and dilation to luminance decreases. This illumination-dependent pupillary modulation has thought to be independent from the top-down influence such as spatial attention. However, it was shown recently that while maintaining central fixation, pupil size is smaller when spatial attention is guided to bright, compared to, dark surfaces, demonstrating the attentional modulation on illumination-dependent pupillary responses. However, the underlying neural mechanism is poorly understood. A large body of research has suggested the close relationship among spatial attention, saccade programming, and working memory, however, the influence of these processes on pupillary light responses is yet explored. Here, we examined the modulation of illumination-dependent pupillary responses by saccade preparation, working memory, and microstimulation of the superior colliculus (SC), a midbrain structure causally involved in shifts of spatial attention and gaze. We hypothesize that enhanced pupillary light responses when the visual (patch) stimulus is presented at the location 1) corresponding to an upcoming saccade destination, 2) activated in spatial working memory, or 3) corresponding to the stimulated region in the SC saccade map. While requiring subjects to maintain central fixation, we presented bright and dark surfaces in two different locations that matched either an upcoming saccade destination (visual delay task), a remembered location (visual memory-guided task), or the stimulated SC site, and another patch was presented at a control location in the opposite visual field. Our results first showed an enhanced illumination-dependent pupillary responses when stimuli were presented at the location corresponding to an upcoming saccade destination or at a location that was activated in memory representation. Moreover, we found that SC microstimulation modulated pupillary light responses in a similar manner, with enhanced illumination-dependent pupillary responses while stimuli presented at the location corresponding to the stimulated SC site. Our results demonstrate that spatial selection for saccade programming and spatial activation in working memory had similar effects on pupillary responses, suggesting the shared neural mechanisms among saccade programming,

working memory, and spatial attention. Furthermore, the SC results provide direct evidence arguing that the SC is mediating this modulation of pupillary light responses.

Disclosures: **C. Wang:** None. **D.P. Munoz:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: European Research Council Grant Agreement No. 647954

Title: Hippocampal synchronization and neocortical desynchronization accompany successful memory formation

Authors: ***B. GRIFFITHS**[1], S. MICHELMANN[1], B. STARESINA[1], M. WIMBER[1], R. CHELVARAJAH[2], D. ROLLINGS[2], V. SAWLANI[2], H. HAMER[3], S. GOLLWITZER[3], G. KREISELMEYER[3], S. HANSLMAYR[1];
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Abstract: An abundance of studies demonstrate a link between episodic memory formation and neural oscillatory activity. However, their findings often conflict with some reporting synchronization, and others desynchronization, in the face of successful encoding. In a step to resolve this contradiction, a recent opinion paper proposed that both are necessary for memory formation (Hanslmayr, Staresina & Bowman, Trends in Neurosciences, 2016). Specifically, synchronized theta and gamma oscillations within the hippocampus serve to bind information into a coherent episode while neocortical low frequency desynchronized activity facilitates information processing relating to the to-be-encoded stimulus. Here, we test the spatial specificity of this hypothesis. We engaged epileptic patients with hippocampal depth electrodes in a typical associative memory paradigm. Patients were presented with either a video or a sound, followed immediately after by a word which they had to vividly associate with the video/sound. Preliminary analysis has revealed hippocampal theta and gamma power increases and neocortical low frequency power decreases (3-20Hz) for later remembered items relative to later forgotten items. These findings support the theory that a synchronized hippocampus and desynchronized neocortex facilitate episodic memory formation.

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Topic: H.02. Human Cognition and Behavior

Support: NSERC - Discovery Grants Program

NSERC CREATE - Biological Information Processing (BIP)

Title: Human memory recall is affected by the phase of low frequency EEG oscillations during stimulus presentations.

Authors: ***A. JALALI**, M. S. TATA, A. J. GRUBER, A. LUCZAK;
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Abstract: Previous studies using electroencephalography (EEG) have shown that low frequency oscillations play an important role in successful memory formation in humans. These studies have linked performance to theta (4-7 Hz) oscillations in awake subjects and slow-wave activity (SWA) during sleep. There is also abundant evidence that memory consolidation is facilitated by slow-wave sleep, in which EEG is dominated by low frequency oscillations. During sleep, the phase of the SWA at the onset of the stimuli affects the recall accuracy in a location memory task. We hypothesized that an analogous phenomenon may take place in the awake state such that the phase of low frequency EEG oscillations at the onset of stimuli will affect memory recall. In order to test our hypothesis, participants performed a word-nonword matching memory task while EEG signals were collected with a 128-channel Electrical Geodesic Incorporated sensor net. The task consisted of four blocks, each with an encoding phase in which novel word-nonword pairs were presented every 5 seconds for 100 seconds (20 pairs total), followed immediately by a test phase in which subjects where shown one of the words and presented with 4 target nonwords. A correct recall occurred when subjects selected the nonword previously paired with the word. We presented every one of the 20 words in series during the test phase. We found that recall performance was higher when the stimuli were presented at a particular phase in the theta and delta bands of EEG collected from the frontal cortex, but not EEG collected from posterior cortical regions. We thus suggest that visual information related to language may be

more readily encoded into short-term memory during particular phases of ongoing cortical neural activity.

Disclosures: **A. Jalali:** None. **M.S. Tata:** None. **A.J. Gruber:** None. **A. Luczak:** None.

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Program#/Poster#: 644.14/LLL14

Topic: H.02. Human Cognition and Behavior

Support: DFG FOR1581

Title: Resting state functional connectivity between hippocampal and prefrontal brain areas is associated with the renewal effect

Authors: ***A. GOLISCH**, L. M. SCHWEIZER, B. GLAUBITZ, M. TEGENTHOFF, S. LISSEK;
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Abstract: Renewal describes the recovery of an extinguished response if the context during retrieval differs from the context of extinction. This context-dependent learning phenomenon is above all mediated by hippocampal activation. In associative learning tasks, participants showing the renewal effect (REN) exhibit higher hippocampus activation during acquisition and extinction compared to participants without renewal (NoREN). Moreover, REN participants also show higher ventromedial prefrontal activation during retrieval than NoREN participants, while NoREN participants exhibit higher activation in dorsolateral prefrontal cortex. Consequently, a connection of the hippocampus with prefrontal areas might be conceivable as a mechanism underlying the renewal effect. In this study we performed both task-based and resting state fMRI at 3T in order to assess brain activation during extinction retrieval. We observed significantly higher hippocampus activation in REN participants. These task-related hippocampal clusters were used as seeds in subsequent seed-based functional connectivity analyses on resting state patterns in REN and NoREN participants. After extinction retrieval REN participants showed stronger hippocampal connectivity with dorsolateral and ventromedial prefrontal brain regions as well as with cingulate gyrus than NoREN participants. Therefore, our data suggest a hippocampal-prefrontal network that is specifically involved in extinction learning and thus mediating the renewal effect. These present findings moreover support previous observations that REN participants use distinct encoding strategies in context-related tasks, which reflect in their brain activation as well as in their connectivity patterns.

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Program#/Poster#: 644.15/LLL15

Topic: H.02. Human Cognition and Behavior

Support: DFG Grant 2653/6-1

Title: Modality-independent differential effects of ongoing EEG beta and theta power on memory formation

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[1]Dept. of Systems Neurosci., Univ. Med. Ctr. Hamburg Eppendorf, Hamburg, Germany; [2]Dept. of Psychology in Educ., Univ. of Muenster, Muenster, Germany

Abstract: Recently, elevated ongoing EEG power in the theta (3-7 Hz) and low beta (13-17 Hz) frequency range before being exposed to a new stimulus has been associated with successful subsequent memory formation for visual stimulus material. However, so far, it has been unclear whether this activity is merely specific to visual processing or whether it reflects a state that generally facilitates the encoding of new information, independent of stimulus modality. To shed light on this issue, the present study investigated the relationship between neuronal pre-stimulus oscillations and verbal memory formation in different sensory modalities. A within-subject design was employed to identify oscillatory states related to successful memory formation for written and spoken words in an incidental encoding task. Further, we investigated whether similar neuronal states promote encoding not only of the stimulus itself, but also of the context in which it is presented, as implemented by a background picture. Results revealed similar EEG activity to be associated with subsequent memory for the stimulus itself in both modalities. Elevated power in the low beta band 800 - 200 ms prior to stimulus onset differentiated successful from failed memory formation for both written and spoken words. In contrast to the stimulus-encoding related effects in the low beta band, increased pre-stimulus theta power was found before successful compared to failed encoding of the associated context. It is suggested that ongoing pre-stimulus beta activity reflects a memory-promoting state and is likely to be moderated by modality independent attentional processes. Ongoing theta power on the other hand is suggested as an indicator of enhanced binding of interlinked information.

Disclosures: **S.L. Schneider:** None. **S. Scholz:** None. **M. Rose:** None.

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Topic: H.02. Human Cognition and Behavior

Support: Penn State Social Science Research Institute Level 2 Grant

Title: Distracted from distraction: interactions of prefrontal and cerebellar regions with the default mode network shield cognition from distraction under high working memory load

Authors: *A. S. WEIGARD, S. J. WILSON, C. HUANG-POLLOCK, Z. SHAPIRO, H. GALLOWAY-LONG;
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Abstract: Human cognitive performance is often disrupted by distractions related to sensory stimuli and affective states, but, paradoxically, a wealth of evidence also suggests that higher working memory load buffers cognitive processing from the impact of distraction. Although the neural mechanisms that mediate this buffering effect are not currently known, it has been posited that regions involved in working memory maintenance and cognitive control inhibit the processing of basic sensory and affective information during high load. However, it is also possible that these regions reduce distraction under high load by suppressing activity in the default mode network (DMN). As the DMN redirects attention from task-relevant processing to rumination and other off-task cognitions, interactions with this network may serve to suppress these cognitions in order to prevent distraction. The current study utilized an aversive odor manipulation to identify neural structures that buffer performance from distraction under high working memory load, and employed context-specific connectivity analyses to determine whether these structures interact with other brain regions related to the DMN or basic sensory and affective processing.
As expected, a significant interaction between distraction conditions and task load demonstrated that response times were impacted by the aversive odor manipulation in a low load (1-back) but not a high load (3-back) working memory task. Areas in the left dorsolateral prefrontal cortex (DLPFC), left ventrolateral prefrontal cortex (VLPFC) and the right cerebellum displayed load by distraction interactions consistent with a role in load-related buffering effects. The VLPFC and cerebellar regions each displayed evidence of context-specific negative connectivity with DMN structures (medial prefrontal cortex and the posterior cingulate / precuneus, respectively). In contrast, there was no evidence for context-specific connectivity with regions that mediate basic sensory and affective information processing.
Results suggest that suppression of DMN-mediated processing is a plausible mechanism though which regions that support cognitive control prevent distraction under high, but not low, working

memory load. Furthermore, they are consistent with developing theories of the role of the cerebellum in cognition which highlight this region's role in coordinating cognitive processes to optimally suit task demands.

Disclosures: A.S. Weigard: None. **S.J. Wilson:** None. **C. Huang-Pollock:** None. **Z. Shapiro:** None. **H. Galloway-Long:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIMH RO1MH60941

Title: Imaging the future: The core episodic simulation network dissociates as a function of timecourse and integration demands

Authors: *P. P. THAKRAL[1], R. G. BENOIT[2], D. L. SCHACTER[1];
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Abstract: Episodic simulation - the construction of a mental representation of a possible future event - is an adaptive function that has been consistently associated with enhanced neural activity in a set of neural regions referred to as the core network. This network includes the medial parietal and prefrontal cortex, medial temporal lobe, including the hippocampus, and lateral temporal and parietal cortex. Much work remains to be done to elucidate the contributions of individual regions within the core network to specific features of episodic simulation. In the current functional magnetic resonance imaging study, we investigated whether regions of the network could be dissociated as a function of the timecourse of their engagement during simulation (i.e., whether neural activity is transient or sustained), and also assessed whether regions of the network vary as a function of the integration demands of a simulated event (i.e., an event with fewer or more components). Participants imagined future events in response to place, person, and object cues. We manipulated the integration demands of the simulation task by varying the number of event components that participants were cued to include in their simulated event (3, 4, or 5). Cues were presented for 5 seconds followed by a variable delay fixation period for 8-12 seconds. Participants were instructed to continually simulate the event throughout the delay period. Transient neural activity was defined as activity specific to the cue period and sustained neural activity was defined as activity associated with the delay period. Relative to a

non-episodic control task, sustained simulation-related activity was identified in the majority of the core network. Additional analyses revealed that activity in the medial parietal and dorsolateral prefrontal cortex was further modulated as a function of integration demands (i.e., 5 > 3). Of particular interest, while the hippocampus was insensitive to integration demands, we observed a temporal dissociation within the hippocampus. Specifically, transient activity was identified in the anterior hippocampus and sustained activity was identified in more posterior hippocampal regions. Consistent with prior theories of hippocampal function, the anterior hippocampal activity may reflect the initial retrieval of gist-based information associated with a simulated event and the posterior activity may reflect further detailed processing of the simulated event. The present findings thus indicate that regions within the core episodic simulation network dissociate as a function of timecourse as well as the integration demands of a simulated event.

Disclosures: **P.P. Thakral:** None. **R.G. Benoit:** None. **D.L. Schacter:** None.

Poster

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Program#/Poster#: 644.18/LLL18

Topic: H.02. Human Cognition and Behavior

Title: Brief electrical stimulation to the human amygdala enhances recognition memory for neutral images

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Abstract: Emotional events are often better remembered than neutral events, and this benefit depends on the amygdala. We have previously demonstrated that brief basolateral amygdala electrical stimulation enhances memory in rodents. This study examined whether human amygdala stimulation immediately following the presentation of neutral object photographs enhanced later recognition memory. We recruited 13 epilepsy patients undergoing intracranial EEG (iEEG) with depth electrode contacts placed in basolateral amygdala and subregions of the hippocampus. During continuous iEEG, each participant was presented a series of photographs of neutral objects, half of which were followed immediately by a unilateral stimulation to the amygdala (8 trains of 50-Hz pulses for 1-second at 0.5 mA after image offset). No epileptiform activity was elicited by the stimulation. Participants reported no awareness of the stimulation. Recognition memory and subjective confidence for half the photographs was tested immediately after the study session and for the other half of the photographs the following day. On the

recognition memory test administered the following day, participants recognized neutral objects initially followed by amygdala stimulation more accurately than control objects. The result was similar when only high-confidence judgments were included. On the immediate recognition memory test, participants performed similarly for both object conditions. These results are similar to the previous results from rodents. The extent of the one-day memory enhancement was inversely correlated with neurocognitive deficits as measured by IQ. Specifically, patients with lower IQ generally showed greater memory enhancement specifically on the one-day test (interaction in stimulation condition by test delay controlling for full-scale IQ). We are also currently investigating the effects of amygdala stimulation on network oscillatory activity, autonomic physiology, and emotional processing. The current results indicate that brief electrical stimulation to the human amygdala can enhance item-specific memory for neutral stimuli even in the absence of awareness of the stimulation, reflecting a key role of the amygdala in prioritizing experiences for long-term storage in declarative memory. Amygdala stimulation likely engages amygdala-hippocampus connections that normally serve to prioritize memory for emotional events and may provide a therapeutic route for patients with memory deficits.

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Topic: H.02. Human Cognition and Behavior

Support: European Union FP7 Marie Curie ITN grant N. 606901 (INDIREA)

Title: Is encoding into visual working memory a parallel process? Evidence from statistical modelling and EEG decoding.

Authors: *E. S. DALMAIJER[1], M. STOKES[1], M. HUSAIN[2];
[1]Exptl. Psychology, [2]Nuffield Dept. of Clin. Neurosci., Univ. of Oxford, Oxford, United Kingdom

Abstract: *Background*
Visual Working Memory (VWM) is a highly limited resource that we use to temporarily store information on items that we encounter. How different items are encoded into VWM remains unclear. Some think individuals can encode multiple items in parallel. An alternative idea is that encoding is serial, with only one item being processed at a time. This notion is fundamental to

the binding problem, and several contemporary models of VWM. However, despite a large body of work, there is little consensus in the existing literature.
Methods
We used a whole report task in which participants were asked to remember a single or two Gabor patches simultaneously. Stimuli were shown for various durations (ranging from 0 to 580ms), and were masked directly after. Participants sequentially indicated the colours they remembered on two continuous colour wheels. On a version of the task with a single exposure duration of 200ms, we recorded electroencephalography (EEG) data that we later subjected to a classification algorithm.
Results
If two items were presented, the first response's accuracy matches that of a single item trial, and the second response is significantly worse. We modelled the distributions of errors as a function of exposure duration, to estimate the probabilities of participants remembering none, only one, or both of the Gabor orientations. This demonstrated that the probability of remembering no item reduces with exposure duration, whereas the probability of remembering both items increased. Crucially, the probability of remembering only one item could be completely predicted by the probability of remembering both. This is in line with a parallel encoding framework.
Discussion
The resolution of an item's representation in VWM increases with encoding duration. Importantly, we demonstrate that this is a parallel process: Multiple items can be encoded at the same time, although not necessarily at the same rate.

Disclosures: **E.S. Dalmaijer:** None. **M. Stokes:** None. **M. Husain:** None.

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Topic: H.02. Human Cognition and Behavior

Support: ESRC 50500.GMGM.RRAI18081

Title: Retrieval as a fast route for consolidation: evidence from decontextualization and semanticization of memory representations

Authors: ***A. C. SANCHES FERREIRA**, M. WIMBER;
Sch. of Psychology, Univ. of Birmingham, Birmingham, United Kingdom

Abstract: Retrieval can act as a powerful memory enhancer. While this observation has been well established in the cognitive literature, the neurocognitive mechanisms underlying such enhancement are still unknown. We here tested the possibility that retrieval solidifies memories through online reactivation mechanisms similar to those involved in offline memory consolidation (e.g. during sleep). If retrieval does indeed depend on neural memory reactivation, one would expect that new episodic memories, initially rich in contextual detail, will become gradually decontextualized as they undergo repeated retrieval, and will transform into gist-like semantic representations. To test these *decontextualization* and *semanticization* hypotheses, we conducted a pattern fMRI study. Participants encoded scene-object pairs, with objects belonging to a number of different semantic categories. They then either retrieved or restudied the objects over two sessions, 48 hours apart. Using representational similarity analysis (RSA), we traced the dynamic changes in item-specific and categorical activation patterns representing each memory in higher order visual areas. Results show that contextual information encoded at study (such as background colour) becomes lost across retrieval repetitions to a greater extent than across restudy ones. Moreover, retrieved (as opposed to restudied) objects become, neurally, less individualised, and more semanticized. Taken together, these findings support the hypothesis that retrieval can act as a fast route to memory consolidation.

Disclosures: A.C. Sanches Ferreira: None. **M. Wimber:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: National Science Foundation of China(NSFC 31571114)

Title: Effects of learning time on brain activation and connectivity during recent and remote associative memory retrieval

Authors: *D. GUO[1], L. ZHAN[1], G. CHEN[2], J. YANG[1];
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Abstract: When stimuli are learned by repetition, they are remembered better and retained for a longer time. However, it is unclear what neural mechanisms underlie the learning effect over time. To address this issue, we explored whether the hippocampus (HPC) and other brain regions

were differentially involved in associative memory after face-scene pairs were learned once or six times. The associative memory was tested at four time intervals, i.e., 20-min, 1-day, 1-week, and 1-month, when participants performed an associative recognition task to judge whether the word pairs were old. The results showed that compared to learning once, learning six times significantly increased the activation in the HPC and posterior cortical regions, and decreased the activation in the orbitofrontal cortex (OFC). In addition, the physiophysiological interaction (PPI) analysis showed that there was stronger correlation between the HPC and parahippocampal place area (PPA) after repetitive learning (vs. learning once), and stronger correlation between the HPC and OFC after learning once (vs. learning six times). In regard to the time change over time, learning once induced the HPC activation decreased and PRC activation increased over time; and learning six times induced the activation of HPC, parahippocampal cortex (PHC) and PPA increased over time. These results suggested that learning experience enhance the involvement of the HPC and posterior regions in associative memory, and there are two different mechanisms for consolidating and retrieving associative memory according to the learning experience.

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Support: NIH Grant R01MH100121

Title: Mapping cortical representations of semantic similarity using Wikipedia and Google News

Authors: ***E. L. ZIPPI**, N. W. MORTON, M. L. MACK, A. R. PRESTON;
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Abstract: Cognition acts on a rich semantic structure that extends beyond visual features, such that concepts are represented in terms of both meaning and perceptual characteristics. While numerous studies have shown that regions within the visual system organize categories according to their perceptual properties, less is known about whether meaning-based representations are present within the same regions. Here, we employ models of semantic similarity derived from Wikipedia and Google News to interrogate whether multivariate patterns in visual regions could be explained by the semantic similarity among stimuli. Participants viewed images of famous people and places during fMRI scanning to estimate neural responses

to each stimulus. Semantic models generated predictions for the relative similarity of each pair of stimuli within visual subcategories (i.e., male and female for faces; manmade and natural for scenes). Two different models were constructed to quantify semantic relationships. The first model estimated semantic similarity based on word co-occurrence in a text-corpus constructed from Wikipedia articles corresponding to each stimulus. Natural language processing tools and dimensionality reduction techniques produced vector representations for each of the articles, from which the cosine between each pair was used to estimate semantic similarity. The second model used representational vectors for each stimulus built from a Google News text-corpus and a continuous bag-of-words algorithm from Google's publically available word2vec. We compared the predictions made by the two models to neural similarity matrices that quantified the pairwise correlation between activation patterns for each stimulus. To separate effects of low-level visual similarity on neural response from those related to semantic structure, we contrasted the correlation between neural response and semantic models with that of the visual models. We found a significant correlation between neural measures of representational similarity within lateral occipital and fusiform cortex and the model-based semantic similarity predictions. In contrast, early visual cortex, lateral occipital, and parahippocampal representations were correlated with predictions from a low-level visual model. In particular, inferior temporal cortex was significantly more correlated with the semantic models than the visual model, suggesting it may play a larger role in semantic representation than visual representation. These findings demonstrate that the rich semantic structure found in web-based text corpora can be leveraged to reveal the neural codes of semantic meaning.

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Topic: H.02. Human Cognition and Behavior

Title: Bistable entropy networks predict human memory formation

Authors: *R. HAQUE[1], A. VAZ[2], R. YAFFE[3], K. ZAGHLOUL[1];
[1]Natl. Inst. of Hlth., Bethesda, MD; [2]Duke Univ., Durham, NC; [3]Johns Hopkins, Baltimore, MD

Abstract: Oscillatory activity in the human cortex has been widely implicated in memory and learning, particularly in the theta and gamma bands. Recent evidence has suggested that much of what is assumed to be oscillatory change may be stochastic volatility or arrhythmic activity. In this study, we recorded intracranial EEG from 33 patients undergoing a paired associates

memory task. For successfully remembered items, we found significant decreases in entropy and significant increases in the range and variance in the right hemisphere. Alternatively, we observed significant increases in sample entropy and significant decreases in range and variance in the left hemisphere. These time-domain metrics also demonstrated better discrimination of the LFP signal than frequency based metrics during successful memory. Given these results, we suggest the exploration of metrics that directly describe LFP distortions rather than those that abstract into the frequency domain.

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Poster

644. Human Cognition and Memory IV

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 644.24/LLL24

Topic: H.02. Human Cognition and Behavior

Support: NYU REF Grant

Title: Representations of spatial priority during nonspatial working memory

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Abstract: It has been established that a several cortical areas encode spatial priority - a weighted representation of space that combines information about stimulus salience and task-relevance signals. An intriguing hypothesis is that these signals, although spatial in nature, may provide the means by which non-spatial feature information can be maintained in working memory (WM). The present study sought to dissociate spatial priority from feature information held in WM to investigate how spatial priority representations may contribute to feature WM. Observers saw 4 sample Gabor gratings - one in each quadrant - and were cued to maintain the orientation of one of these gratings in WM. After a delay, a second cue indicated the quadrant in which the test grating would appear. After a second delay, the test grating appeared, and observers indicated whether the grating matched the orientation of the original sample grating. Critically, during the second delay, retrospective priority for the spatial location of the sample grating and prospective priority for the spatial location of the test grating are dissociated.
Individual observers' population receptive fields (pRFs) were estimated using fMRI in an independent scan. pRF maps were then used to reconstruct estimates of spatial priority during the

second delay period. In visual area V2, reconstructions demonstrated a persistent representation for the original (i.e., currently irrelevant) sample location. This finding suggests that feature WM involves retrospective representations of the spatial location at which the feature was initially encoded. In contrast, reconstructions from parietal region IPS0 demonstrated a representation of the upcoming probe location. Together, these results demonstrate an interrelationship between spatial priority and feature WM, and indicate that multiple representations of spatial priority may be required for the independent maintenance of features and spatial priority.

Disclosures: **M. Rahmati:** None. **M.J. Payton:** None. **T.C. Sprague:** None. **C.E. Curtis:** None. **K.K. Sreenivasan:** None.

Poster

645. Reward Learning in Humans

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 645.01/LLL25

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant 2R01MH063760

Title: Different paths to the same destination: information-integration category learning with and without feedback

Authors: *Y.-W. WANG, V. V. VALENTIN, F. G. ASHBY;
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Abstract: Information-integration (II) categorization is believed to be a procedural skill. Evidence suggests that II learning is mediated by the basal ganglia, and is best acquired via immediate feedback. Since humans have multiple learning systems, an important question is whether II learning is also possible by other brain systems. Thus, the present study investigates whether II learning is possible in the absence of feedback. Although several previous studies reported that II observational learning (OL) was impaired relative to feedback learning (FL), these conclusions were drawn by comparing average scores in the OL and FL conditions, which obscures individual differences. We studied these issues more closely using a new OL paradigm, which was similar to a series of A/not A tasks. Previous research showed that Parkinson's disease patients seem to learn A/not A categorization normally, thus it might provide a way of learning without mediation of basal ganglia, although none of the A/not A studies had used II categories to our knowledge. Specifically, in our OL condition, participants were asked to memorize figures from one of two categories in each block, and to categorize figures without receiving any feedback at the end of each block; in our FL condition, figures from both categories were shown

in a randomly mixed order, and participants were asked to categorize the figures and would receive correct or incorrect feedback on each response. We compared the ratio of successful learners and unsuccessful learners, as well as learning curves of successful learners in both conditions, and found two major results: 1. Learning curves of successful learners in OL and FL did not show significant differences; 2. fewer participants successfully acquired optimal strategies in OL than in FL. Our findings suggest that II learning might be possible in some participants without mediation of the basal ganglia. Instead, we propose that for these successful participants, the cortical-striatal-cortical circuits that mediate feedback-based II learning are replaced by direct cortical-cortical circuits that bypass the basal ganglia.

Disclosures: **Y. Wang:** None. **V.V. Valentin:** None. **F.G. Ashby:** None.

Poster

645. Reward Learning in Humans

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 645.02/LLL26

Topic: H.02. Human Cognition and Behavior

Support: NSF Career: #1351748

Pennsylvania Department of Health's Commonwealth Universal Research Enhancement Program #SAP4100062201

Title: Long-term sequence training alters movement representations in primary motor cortex

Authors: *P. BEUKEMA[1,2], T. VERSTYNEN[3,2];
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Abstract: Efficient production of a sequence requires binding temporally independent movements into single, unified action chunks. This process should be reflected by increases in the similarity of each movement's response times (i.e, increased correlation in temporally adjacent response speeds) as well as a shift in the neural representations of each action. We trained human subjects (Sequence Group, N=9, 6 male) to perform a 32-item sequence of finger movements across 25 training sessions (5 weeks). The sequence was constructed such that some finger combinations exhibited higher frequency pairings (e.g. index followed by ring) than others. A second group (Control Group; N=9, 6 male) underwent the same 25 training sessions but instead performed pseudorandomly ordered movements on each day without exposure to the sequence. A hierarchical agglomerative clustering algorithm applied to reaction times of each

element in the 32-element sequence revealed a well ordered clustering structure of the trained movements in the Sequence group, with distances between successive elements in the dendrogram being decreased following training. As expected, the Control group exhibited no significant distance changes in their clustering structure during the same trial blocks. The binding of sequence elements observed in the behavioral data should also be reflected as concomitant changes in the movement representations in primary sensorimotor areas. To assess this, the cortical representations of each effector were mapped before and after training using fMRI (Siemens Verio 3T scanner,32 channel head coil, TR: 2000ms, TE: 30.0ms, MBAF, 3, 66 slices). During each scan, subjects performed each of the four finger movements separately to facilitate isolation of the motor representations controlling each finger using representational similarity analysis (Kriegeskorte et. al., 2008). In the Sequence group, a non-parametric permutation test searchlight revealed regions along the anterior central sulcus with significant decreases in representational distances following training that were qualitatively different in both magnitude and location compared to the Control group. These results suggest that movement representations reorganize with training to facilitate binding of independent actions into unified chunks.

Disclosures: P. Beukema: None. **T. Verstynen:** None.

Poster

645. Reward Learning in Humans

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 645.03/LLL27

Topic: H.02. Human Cognition and Behavior

Support: NIH Training Grant 5T90DA022761-10

PA Dept. of Health Formula Award SAP4100062201

Title: Neural substrates of risky spatial decisions under conditions of perceptual uncertainty

Authors: *K. JARBO, T. D. VERSTYNEN;
Psychology, Carnegie Mellon Univ., Pittsburgh, PA

Abstract: To maximize expected gain and avoid losses on visually guided spatial decisions (Trommershäuser, Maloney, & Landy, 2008) a global network of cortical and subcortical brain regions integrates spatial uncertainty (Gottlieb & Balan, 2010) with costs and expected reward (Hsu, Bhatt, Adolphs, Tranel & Camerer, 2005) to bias selections of a target away from a penalizing distractor. Using a visually guided spatial decision-making task, we examined how

sensory uncertainty (low vs. high target variance) and cost (no penalty vs. penalty) interact when making spatial decisions. Across three consecutive sessions (two behavioral, one MRI), participants (N=20) used a mouse to maximize their point total across several hundred trials by attempting to select the center of a spatially presented target. A distractor was presented adjacent to the target and, on some trials (penalty trials) points were lost based on the proximity of the selection to the center of the distractor. Behaviorally, participants avoided the distractor both when losses were incurred and when target estimation was less certain. While selection endpoints were also more variable under high target variance, a significant interaction between variance and penalty biased selections away from the distractor more strongly than either main effect. Functionally, we identified task-related activity in a broad network of interconnected cortical and subcortical regions that correlated with variability in selection bias. Distributed clusters of significant activity (all $t(19)s > 4$, FDR-corrected $q = 0.01$) were present in orbitofrontal, dorsolateral prefrontal, and posterior parietal cortex as well as distinct dorsomedial regions within the caudate and putamen, bilaterally. These cortical regions match a previously identified structural network thought to support the integration of reward and spatial attention during decision-making (Jarbo & Verstynen, 2015). Together, these results identify a plausible neural substrate that allows for integrating estimations of risk with perceptual uncertainty during spatial decisions.

Disclosures: K. Jarbo: None. **T.D. Verstynen:** None.

Poster

645. Reward Learning in Humans

Location: Halls B-H

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Program#/Poster#: 645.04/LLL28

Topic: H.02. Human Cognition and Behavior

Support: NSF CAREER Award #1351748

Title: A biologically-constrained hybridization of reinforcement learning and accumulator models for adaptive decision-making

Authors: *K. E. DUNOVAN[1,2], T. VERSTYNEN[3,4];
[1]Psychology, [2]Ctr. for the Neural Basis of Cognition, Univ. of Pittsburgh, Pittsburgh, PA;
[3]Psychology Dept., [4]Ctr. for the Neural Basis of Cognition, Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Cognitive process models, such as reinforcement learning (RL) and accumulator models of decision-making, have proven to be highly insightful tools for studying adaptive

behaviors as well as their underlying neural substrates. Currently, however, two major barriers exist preventing these models from being applied in more complex settings: 1) the assumptions of most accumulator models break down for decisions involving more than two alternatives; 2) RL and accumulator models currently exist as separate frameworks, with no clear mapping between trial-to-trial learning and the dynamics of the decision process. Recently we showed how a modified accumulator model, premised off of the architecture of cortico-basal ganglia pathways, both predicts human decisions in uncertain situations and evoked activity in cortical and subcortical control circuits (Dunovan et al. 2015). Here, we present a synthesis of RL and accumulator models motivated by recent evidence that the basal ganglia acts as a site for integrating trial-wise feedback from midbrain dopaminergic neurons with accumulating evidence from sensory and associative cortices. We show how this hybrid model can explain adaptive, multi-alternative (2- & 4-choice) decisions in a computationally efficient manner. More importantly, by parameterizing the model to conform to various underlying assumptions about the architecture and physiology of basal ganglia pathways, model predictions can be rigorously tested against observed patterns in behavior as well as neural recordings. The result is a biologically-constrained and behaviorally tractable description of trial-to-trial learning effects on decision-making among multiple alternatives.

Disclosures: K.E. Dunovan: None. **T. Verstynen:** None.

Poster

645. Reward Learning in Humans

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Program#/Poster#: 645.05/LLL29

Topic: H.02. Human Cognition and Behavior

Support: Pennsylvania Department of Health's Commonwealth Universal Research Enhancement Program #SAP4100062201.

Title: Sensory uncertainty influences value-based risky decisions

Authors: *R. M. FLEMMING[1], K. JARBO[2], T. D. VERSTYNEN[2];
[1]Neuroscience, Psychology, Univ. of Pittsburgh, Pittsburgh, PA; [2]Psychology, Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Many value-based decisions, such as gambling on a risky choice with a potentially high reward, are contingent on sensory signals and feedback from past choices; however, it remains unclear how sensory evidence may impact these types of risky decisions. Using a spatial decision-making task, we manipulated sensory uncertainty (i.e., spatial variance), value

magnitudes, and probability of punishments, we examined how the reliability of a stimulus biases feedback-based risky decisions. Two target stimuli were simultaneously presented (300ms duration) as two Gaussian distributions of dots with means separated by 100 to 200 pixels and spatial variances that could be increased to manipulate sensory uncertainty. In 800 trials, subjects (N = 20) used a mouse to select the center of either a Risky Target that had a specified probability of large reward or could incur a comparable penalty, and a Stable Target, which yielded consistent smaller rewards, but varied in spatial uncertainty across trials. Feedback was based on the selection endpoint distance from the center of the chosen target. Given the choice between the two targets, subjects began to choose the Risky Target more frequently ($F(3,57) = 43.8366$, $p < 0.001$) when the reward to penalty ratio was greater than 2:1. Subjects also chose the Risky Target more frequently when the spatial uncertainty of the Safe Target was high ($F(3,57) = 24.9440$, $p < 0.001$), resulting in a significant interaction ($F(9,171) = 4.6506$, $p < 0.001$) between feedback signals and spatial uncertainty on risky decisions. Our results show that risky choices become more viable as the perceptual uncertainty of the "safe" choice is increased relative to a risky alternative.

Disclosures: **R.M. Flemming:** None. **K. Jarbo:** None. **T.D. Verstynen:** None.

Poster

645. Reward Learning in Humans

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Program#/Poster#: 645.06/LLL30

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Support: ERC Grant 2010-AdG_20100407

DFG Grant SFB TRR 58

DFG Grant SFB 936

Title: Rapid learning of the value of choices from delayed feedback during navigation in humans: computational and neural mechanisms

Authors: ***G. WIMMER**[1,2], S. SCHÖNIG[2], L. AUSTERMANN[2], L. MEISSNER[2], M. HEBART[3,2], C. BÜCHEL[2];
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Abstract: Rewards exert a strong influence on learning and decision making. While dopaminergic and striatal mechanisms can only support reinforcement learning when feedback occurs within several seconds of predictive events, many salient events in life are preceded by sequences of events and actions that occur over significantly longer timescales. To account for learning from these common situations where feedback is delayed, alternative computational models and neural mechanisms are necessary. In the current experiments, we tested whether candidate models, including eligibility traces, forward planning, and post-feedback replay can account for behavioral performance and neural activity during learning. In our experiments, participants navigated temporally extended sequences of three unique rooms (states), leading to a reward or loss up to 50 seconds later. Each sequence was followed by a 15 sec rest period. In order to examine early learning, the task included 8 intermixed sequences that were only repeated 4 times. Behaviorally, participants exhibited acquisition of optimal choices after only one or two experiences. Further, in a separate experiment, for a subset of sequences only experienced once, participants were reliably able to identify whether a start state led to a reward or loss. These results demonstrate rapid learning across long delays that cannot be accounted for by traditional striatal learning mechanisms. In our fMRI study, we utilized specific visual categories of stimuli in each state of navigation (faces, scenes, objects, and motion). This allowed us to use multivariate classification and representational similarity analyses (RSA) to test for predictive effects related to forward planning as well as maintenance-related effects related to eligibility and replay during rest breaks. Our behavioral and neural results provide insight into how the brain can rapidly learn from and use delayed reward feedback in later decision making.

Disclosures: **G. Wimmer:** None. **S. Schönig:** None. **L. Austermann:** None. **L. Meissner:** None. **M. Hebart:** None. **C. Büchel:** None.

Poster

645. Reward Learning in Humans

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 645.07/LLL31

Topic: H.02. Human Cognition and Behavior

Support: SFB TRR135

Title: Visual salience effects on reinforcement learning

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[1]Biol. Psychology, Justus-Liebig Univ., Gießen, Germany; [2]Inst. of Physics, Chemnitz Univ. of Technol., Chemnitz, Germany

Abstract: Highly salient visual stimuli can draw attention as evidenced by gaze behavior. It has been suggested that reward learning is enhanced for these stimuli, but the underlying mechanisms are yet unclear. Pupil responses, which are linked to locus coeruleus activity, have been proposed to reflect surprise, thereby providing an index of learning. In the current eye-tracking study, we investigated the role of visual salience in a probabilistic reinforcement learning task. On each trial, participants chose from a circular array with three neutral everyday objects and received feedback on the reward outcome. Visual contrast (low/high) and monetary reward probability (low/high) varied between objects. Visual salience influenced attentional allocation: While high-reward stimuli were fixated earlier and with higher probability than low-reward stimuli, this effect was enhanced when salient stimuli were rewarded and attenuated when salient stimuli served as low-reward distractors. Learning, in contrast, was improved when salient stimuli were rewarding but not impaired by salient distractors. Participants were more likely to choose a salient than an equally rewarding non-salient object, both for low and high reward probabilities. The pupil response to reward feedback was decreased on trials when a salient stimulus compared to a non-salient stimulus was rewarded, suggesting that visual salience strengthened participants' expectations regarding reward outcomes. Taken together, our findings suggest that reinforcement learning is biased by attentional mechanisms.

Disclosures: J. Schomaker: None. **W. Einhäuser:** None. **B.C. Wittmann:** None.

Poster

645. Reward Learning in Humans

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Program#/Poster#: 645.08/LLL32

Topic: H.02. Human Cognition and Behavior

Support: NSERC

Title: The sensitivity of Reward Positivity to prediction errors at 2 levels of hierarchy

Authors: *D. SHAHNAZIAN, C. B. HOLROYD;
Psychology, Univ. of Victoria, Victoria, BC, Canada

Abstract: Principles of hierarchical reinforcement learning (Botvinick, Niv, Barto, 2009) implicate learning at different levels of hierarchy from different sources of information. To learn at different levels of hierarchy, an agent has to calculate prediction errors associated with each level of hierarchy. With electroencephalogram, prediction errors correlate with a frontocentral event-related potential occurring 240-340 ms post-feedback, termed reward positivity. This component is posited to originate in the anterior cingulate cortex. The hierarchical reinforcement

learning theory of anterior cingulate cortex (Holroyd & Yeung, 2012) raises the possibility that this brain region is sensitive to reward prediction errors at multiple levels of hierarchy. To investigate whether the reward positivity is sensitive to prediction error at two levels of hierarchy, we recorded electroencephalogram (EEG) from human participants engaged in two-level hierarchical gambling task modified from Diuk et al. (2013). In this task, performance is improved if learning happens at both levels of the hierarchy. We found that the reward positivity is sensitive to reward information at both levels of hierarchy. This finding is consistent with an involvement of the anterior cingulate cortex in hierarchical reinforcement learning.

Disclosures: D. Shahnazian: None. **C.B. Holroyd:** None.

Poster

645. Reward Learning in Humans

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Program#/Poster#: 645.09/LLL33

Topic: H.02. Human Cognition and Behavior

Support: EXC1086

BMI-Bot

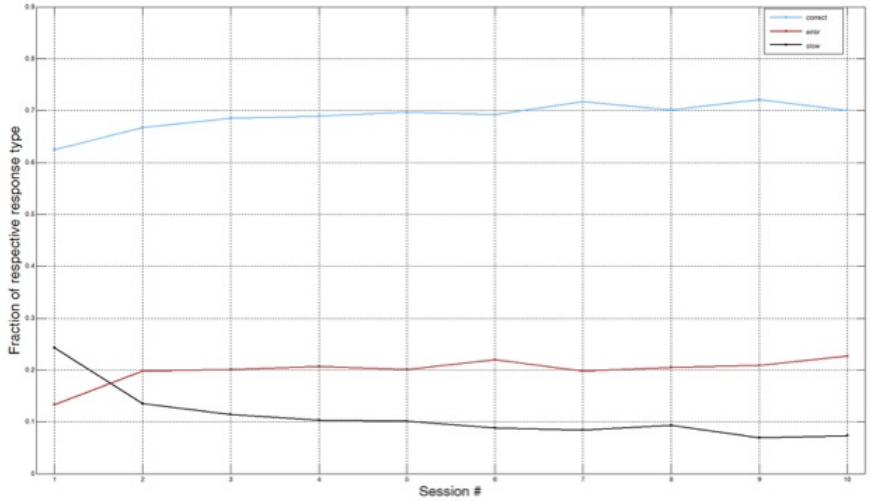
Title: The success of learning from errors: the role of high-gamma EEG and peripheral physiological responses

Authors: *S. S. BERBERICH[1], M. VOELKER[1], L. FIEDERER[1], E. ANDREEV[1], S. CONTZEN[1], A. SCHULZE-BONHAGE[2], W. BURGARD[3], T. BALL[1];
[1]iEEG and Brain Imaging Group, [2]Epilepsy Ctr., Univ. Med. Center, Freiburg, Freiburg, Germany; [3]Dept. of Computer Sci., Albert Ludwigs University, Freiburg, Freiburg, Germany

Abstract: How humans learn from errors is a matter of great interest in neuroscience, yet many aspects of the underlying brain mechanisms and the role of peripheral physiological processes in error-based learning still remain unclear.
Here, we investigated error-related learning in 30 healthy subjects during a classical Eriksen flanker task. Subjects had to use either the left or right hand to respond to the visual stimuli and were instructed to avoid errors in the form of "slow" trials exceeding a pre-defined time limit. EEG data were acquired with a 128-channel system optimized for detection of high-frequency EEG components. To ensure an optimal signal-to-noise ratio, recordings took place in an electromagnetically shielded cabin and with full optical decoupling of all devices. We acquired peripheral physiological data using a high resolution binocular eye tracker and a two-channel

ECG and computed the individual heart rate variability as a parameter previously implicated in learning processes.
Consistent with the instruction stressing the importance of speed over accuracy, the number of slow trials decreased during the experiment (Fig. 1A). Further, we could show a significant post-error heart-rate deceleration, while pupil size was significantly bigger following error trials compared to correct ones. Both parameters showed an opposite yet similar temporal profile (Fig. 1B). In the EEG data, we found high-gamma band (HGB) spectral power increases in the frequency range up to 120 Hz approx. 100 ms post-error and over the frontal midline region. Across subjects, the amplitude of the individual frontal-midline HGB response was significantly correlated with the individual learning performance, as well as the heart rate variability and error-related pupil size response.
Our findings reveal a novel response pattern consisting of central (frontal midline HGB) and peripheral (pupil size, heart-rate variability) components predictive of the individual success in learning from errors and thus open up a new window on the mechanisms contributing to learning via the error-monitoring network.

A) Average fraction of response types



B) Relative pupil size and heart rate during the averaged course of a trial

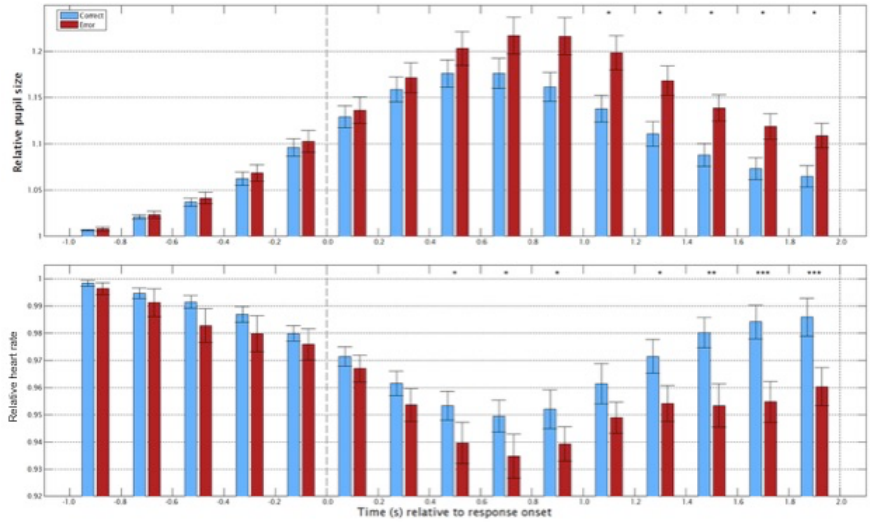


Fig. 1:

A) Average fraction of response types per session for all 30 subjects. Correct trials are indicated by the blue line. The red line shows error trials due to choice of the incorrect hand, while error trials due to exceedance of the time limit are marked by the black line.

B) Relative pupil size and heart rate during the averaged course of error (red) and correct (blue) trials for all 30 subjects. Trial cutting was locked to response onset. Significant differences are marked with an asterisk. Response onset is marked by the left dotted vertical line. The right dotted vertical line indicates feedback onset.

Disclosures: **S.S. Berberich:** None. **M. Voelker:** None. **L. Fiederer:** None. **E. Andreev:** None. **S. Contzen:** None. **A. Schulze-Bonhage:** None. **W. Burgard:** None. **T. Ball:** None.

Poster

645. Reward Learning in Humans

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 645.10/LLL34

Topic: H.02. Human Cognition and Behavior

Support: Graduate School of Robotics Freiburg

EXC1086 BrainLinks-BrainTools

BMI-Bot

Title: Detection of error-related high-gamma activity in non-invasive and intracranial eeg

Authors: *M. VÖLKER[1,3,4,5], S. BERBERICH[1,6], L. D. J. FIEDERER[1,7,5,8], E. ANDREEV[1,10], S. CONTZEN[1,7], A. SCHULZE-BONHAGE[2,8,5], W. BURGARD[4,9,5], T. BALL[1,8,11,5]; [1]iEEG and Brain Imaging Group, [2]Epilepsy Ctr., Univ. Med. Ctr., Freiburg, Germany; [3]Grad. Sch. of Robotics, Albert Ludwigs Univ., Freiburg, Germany; [4]Dept. of Computer Sci., [5]BrainLinks-BrainTools, [6]Fac. of Med., [7]Fac. of Biol., [8]Bernstein Ctr., [9]Autonomous Intelligent Systems, Albert Ludwigs Univ., Freiburg, Germany; [10]Dept. of Informatics, Karlsruhe Inst. of Technol., Karlsruhe, Germany; [11]Epilepsy Ctr., Univ. Med. Ctr. Freiburg, Freiburg, Germany

Abstract: The nature of human error processing and the underlying cerebral mechanisms are of great interest in neuroscience, and error-related brain responses may provide information useful to improve the accuracy of brain-machine interfaces (BMIs). So far, many studies focused on error-related responses below 30 Hz. We were interested in error-related effects in the high-gamma band (HGB, 50-150 Hz), as this frequency range is thought to reflect local cortical information processing more directly than lower frequencies.
30 healthy subjects performed a flanker task as classically used to study error processing (Gehring et. al, 1993). Under time pressure, the subjects had to use the left or right index finger to respond to the respective flanker. Recordings of 128-channel EEG were acquired within an optimized setting for non-invasive EEG high-gamma mapping in an electromagnetically shielded cabin with full optical decoupling of all devices and high-resolution binocular eye tracking. Additionally, 3 patients with pharmacoresistant epilepsy and implanted stereo-EEG (SEEG) electrodes performed the identical task.
As our main result, we show for the first time error-related HGB spectral power modulations up to 120 Hz in non-invasive EEG (Fig. 1A), which could also be used for decoding on a single-trial basis. Additional SEEG data (Fig. 1B) revealed possible sources of these effects, including the premotor cortex. Additional responses were seen in areas not reflected in the non-invasive EEG, such as hippocampus and insula. Importantly, based on the eye-tracking data, we show that the error-related effects in the gamma range cannot be explained by differences in eye movements

including micro-saccades, which had a different spatial distribution compared to the error-related effects.

Our findings open a new window for investigation of the complex neuronal processes related to error perception and ensuing behavioral adaptation. Furthermore, the decodability of error-related HGB responses indicates that these novel error-related brain signals may also be useful for BMI applications.

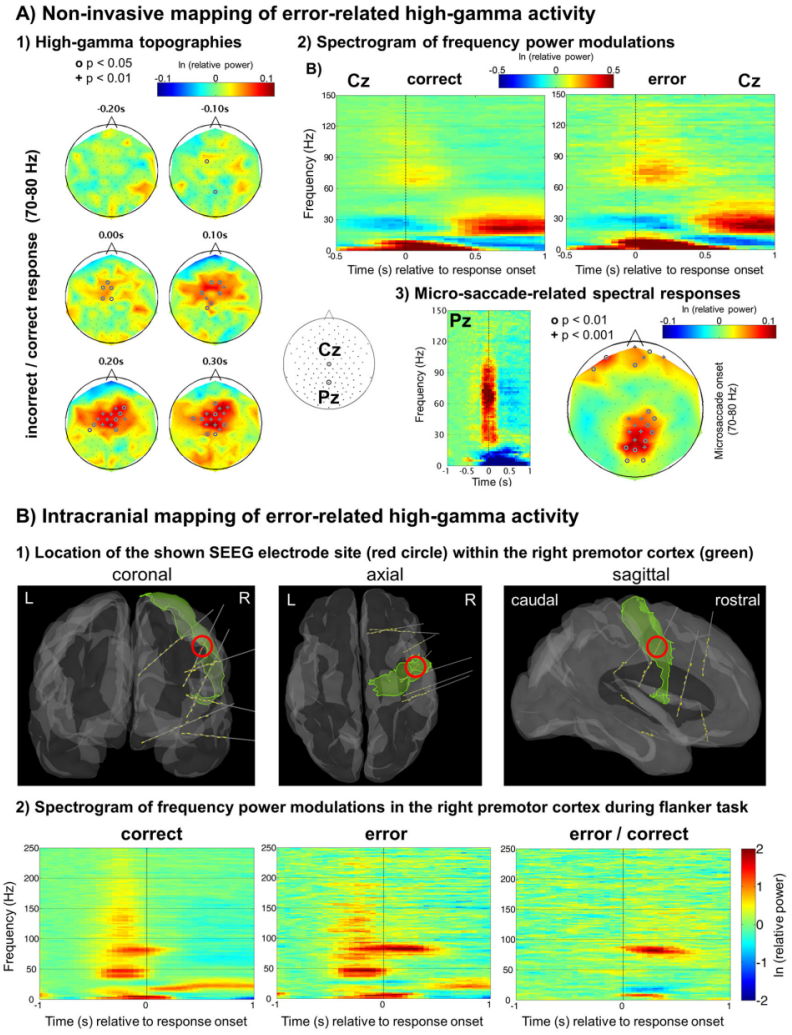


Fig. 1 : Mapping of error-related high-gamma activity in non-invasive and intracranial EEG
A) Topography (1) and spectrogram (2) of error-related high-gamma activity measured with non-invasive EEG in 30 healthy subjects. The high-gamma power increase was significantly stronger in false responses with a spatial focus on fronto-central sites. In comparison, saccade-related spectral responses (3) are distributed frontopolar and centro-parietal.
B) Error-related high-gamma responses in stereo EEG (bipolar re-referencing). The electrode location was close to cortex surface of the right premotor cortex (1). Spectrograms (2) show a broadband gamma power increase prior to movement onset in both correct and false responses, followed by a pronounced high-gamma peak between 70-90 Hz only in incorrect responses.

Disclosures: **M. Völker:** None. **S. Berberich:** None. **L.D.J. Fiederer:** None. **E. Andreev:** None. **S. Contzen:** None. **A. Schulze-Bonhage:** None. **W. Burgard:** None. **T. Ball:** None.

Poster

645. Reward Learning in Humans

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 645.11/LLL35

Topic: H.02. Human Cognition and Behavior

Support: NIMH R01MH109177

NIMH 5T32MH019524

NSF CRCNS 1207833

Title: Humans cache multi-step stimulus expectancies in reinforcement learning

Authors: *E. RUSSEK[1], I. MOMENNEJAD[2], M. M. BOTVINICK[3], N. D. DAW[2], S. J. GERSHMAN[4];
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Abstract: Contemporary theories of decision making suggest human choice evaluation reflects an influence of model-based (MB) and model-free (MF) strategies, associated with distinct neural systems. Whereas MB simulates the sequence of states that will follow a choice and sums their rewards, MF caches future reward estimates. In two studies we employed varieties of revaluation to test the hypothesis that humans also engage in a third strategy, the successor representation (SR). SR caches predictions of which states will follow a choice, aggregating over multiple steps, but can flexibly alter the rewards paired with those states. Each trial of Study 1 (N = 58) had two phases. In a training phase, subjects were exposed to two three-state sequences, rewarded at terminal states. In a revaluation phase, subjects were exposed to a switch in either the reward paired with each terminal state (reward revaluation) or the terminal state to which each middle state transitioned (transition revaluation). Before and after revaluation, subjects indicated their relative preference for visiting the initial state of either sequence. While subjects showed revaluation in all conditions, they were relatively better following reward compared to transition revaluations ($p<.005$). This asymmetry suggests an influence of SR in addition to MB and MF strategies. SR, unlike MF, can adapt rapidly to changes in reward structure by updating reward predictions. However, unlike MB, SR does not represent one-step transitions and cannot adapt rapidly when they are changed. Each block of Study 2 (N = 88) started with a training phase in which subjects made a choice to transition to a second-level state, then a second choice to transition to a rewarding terminal state. In a revaluation phase, subjects experienced reward and transition revaluations, and a novel revaluation in which learning about a terminal-state reward change caused a change in their second-level choice preference (policy revaluation). Caching prevents the SR from altering predicted future states to those implied by the new

second-level choice preference. Although subjects were able to alter stage 1 choices in all conditions, they were relatively better at reward revaluations compared to both transition ($p<.005$) and policy ($p<.02$) revaluations. These results suggest use of intermediate caching strategies, between MB and MF, that trade off flexibility with computational efficiency. Ongoing fMRI investigation of Study 1 seeks neural evidence of SR-prescribed caching. Using category-specific visual responses, we ask whether transition revaluation failures are accompanied by neural evidence of cached multi-step state predictions.

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Poster

645. Reward Learning in Humans

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 645.12/LLL36

Topic: H.02. Human Cognition and Behavior

Support: NIH/NINDS Grant R01NS078784

Title: Disentangling the contributions of episodic memory and incremental learning to value-based decisions

Authors: *K. D. DUNCAN[1], R. T. GERRATY[2], B. B. DOLL[2,4], N. D. DAW[5,4], D. SHOHAMY[2,3];
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Abstract: Learning from past choices and their outcomes guides adaptive decisions. Major advances have been made in understanding how such learning is accomplished over the course of repeated experiences with a fixed set of options, a process well described by reinforcement learning models. Many choices, however, are made between options with which we have sparse experience. Less is known about the cognitive and neural mechanisms by which single episodes are retrieved to support decisions and how this process interacts with incremental learning. We hypothesized that choices based on single past episodes are likely to depend on episodic memory mechanisms in the hippocampus, which are dissociable from striatal-dependent incremental value learning mechanisms. To test this hypothesis, we developed a task in which value-based decisions can be made using both episodically and incrementally learned values. On each trial, participants chose between two cards to win money. Participants could base choices on two

separate features: the color of the 'deck' (blue or red) or the picture of an object on the card (different for each card). The outcomes for each deck were probabilistic and varied slowly over time, allowing participants to incrementally learn which deck had a greater probability of reward. Separately, each object perfectly predicted the card's value, allowing participants to use their memory of a single previous trial to increase winnings. Critically, the value of the deck vs. the objects was not correlated (r=.07), allowing us to track the influence of each type of value on a trial-by-trial basis. This opens the door to the behavioral and neural characterization of each form of learning and their interaction. We found that participants' choices were best predicted by a model that included both incremental learning of deck-values, estimated using a Q-learning algorithm, as well as one-shot episodic memory for object values. Moreover, the strength of episodic memory learning was modulated by the uncertainty of deck values: participants were more likely to use object values from trials that occurred when the deck value was least certain. This effect was not driven by changes in reaction time during object value learning and deck uncertainty did not influence the use of object values, suggesting that uncertainty did not affect participants' attention to objects. These results demonstrate that both incrementally learned values and one-shot memory for distinct episodes guide value-based choices and that their relative contributions are modulated by uncertainty.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: 711 HPW, Chief Scientist Seedling Funds

Title: Examination of the effectiveness of transcranial direct current stimulation (tDCS) to augment cognitive bias mitigation training

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Abstract: Cognitive bias problems are seen in many professions where analysis is an important component (e.g., intelligence, law enforcement, medicine, aviation, journalism, and scientific

research). When an intelligence problem invokes these cognitive biases, analysts may draw inferences or adopt beliefs that are logically unsound or not supported by evidence. Cognitive biases in analysis tend to increase with the level of uncertainty, lead to systematic errors, filter perceptions, shape assumptions and constrain alternatives. The Sirius Program produced computer games to train participants and measure their proficiency in recognizing and mitigating the cognitive biases that commonly affect all types of intelligence analysis. Recent research has demonstrated that transcranial direct current stimulation (tDCS) can accelerate learning and enhance declarative memory (e.g. McKinley, et al., 2013) and can affect the medial prefrontal cortex (mPFC) neural response to making a mistake (Reinhart & Woodman, 2014). A spike of negative voltage that originates from this area of the brain milliseconds after a person recognizes a mistake reinforces the brain to learn and retain information from errors. The hypothesis is that interaction with a video game with constant error feedback, along with tDCS stimulation could lead to significantly more effective training and measurably increased retention of the bias. A total of 21 male and female active duty Air Force volunteers participated in the experiment. All participants were given a test to assess their level of cognitive bias. Next, they played the cognitive bias training game while receiving real (n=10) or sham (n=11) tDCS at 2mA over mPFC. They then completed a post-training test to assess their cognitive bias. All participants returned 1 week later for a follow-up test. The results suggest that the sham tDCS group significantly reduced their level of cognitive bias after training and this effect remained 1 week later. However, the cognitive bias scores for the tDCS group did not significantly change. Because of the mPFC's involvement in learning from mistakes and retrieval of recent and remote memories, it was anticipated that stimulation of this region would enhance the cognitive bias training. However, the data suggest that tDCS interfered with the training and prevented participants from learning to mitigate their cognitive biases. Tronel and Sara (2003) showed that interfering with the mPFC following learning disrupted recall. Hence, it is possible that the tDCS paradigm applied herein augmented the natural activity of the mPFC in a way that impaired encoding or retrieval of the information presented.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH grant R01MH098861

Title: Hemodynamic response function for prediction errors in the ventral striatum

Authors: *G. B. HERMSDORFF[1], Y. NIV[2];
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Abstract: Recent years have seen a proliferation of studies in which computational models are used to specify a set of hypotheses regarding learning and decision-making in humans, which are then tested against data from fMRI. Much of this model-based fMRI effort focuses on the nucleus accumbens or ventral striatum (VS), where the blood oxygenation level dependent (BOLD) response has been shown to reflect reward prediction-error signals putatively from dopaminergic afferents. To make sensible inferences about neural activation in this area from fMRI data, it is important to accurately model the hemodynamic response function (HRF) of the VS, i.e., the hemodynamic response evoked by a punctate neural event in the VS. A canonical HRF, mapped for sensory cortical regions, is commonly used for analyzing activity throughout the brain despite the fact that hemodynamics are known to vary across regions. Here we use data from an experiment focused on learning from prediction errors to fit a VS-specific HRF function. Our results show that the VS-specific HRF differs significantly from the canonical HRF, most importantly peaking at 6 sec after the event, rather than at 5 sec as in the canonical HRF. We demonstrate the superiority of the VS HRF in modeling prediction-error data by showing that using the new VS-HRF increases statistical power. This result is particularly relevant to fMRI studies in neuroeconomics and reinforcement learning as many of these rely on fine-grained analysis of VS BOLD activity to distinguish between important but subtle differences in computational models of learning and choice. We therefore recommend the use of this VS-specific HRF for future fMRI studies of the VS. More broadly, our study highlights the importance of the HRF model used in determining the significance of the results obtained in classical univariate fMRI analysis.

Disclosures: G.B. Hermsdorff: None. **Y. Niv:** None.

Poster

645. Reward Learning in Humans

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Program#/Poster#: 645.15/LLL39

Topic: H.02. Human Cognition and Behavior

Title: Reward expectation in the human sub-thalamic nucleus

Authors: *J. R. FLYNN[1], H. Z. SHOUVAL[2], A. FENOY, Jr.[3];
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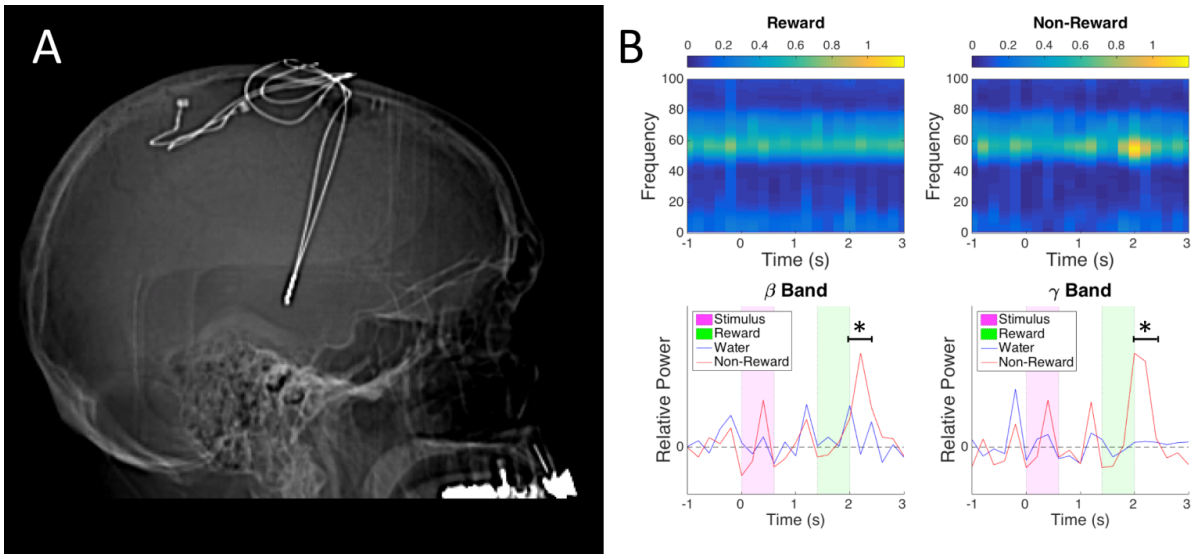
Abstract: The ability to make predictions about the environment is critical for an organism's survival, and the reward system in mammals is critical to this ability. Various reward centers have been shown to project to the sub-thalamic nucleus (STN), and recent evidence in animal models suggests that the region is involved with reward expectation. Our experiments extend these results and specifically test for reward expectation in the human STN.
Parkinson's Disease patients (n=11) were asked to participate in our study during a deep brain stimulator (DBS) implantation procedure (see Fig 1A). During this procedure, recording microelectrodes were implanted within the ventral STN. Patients were presented with a visual stimulus of a green dot, followed one second later by either a reward (32 trials) or nothing (8 trials). The reward stimulus was sugar water administered orally via a syringe pump. For additional controls, a set of 20 trials was performed where patients were simply given sugar water at random with no visual stimulus. The reward/non-reward conditions were analyzed and compared using the recorded LFP data.
Our analysis has shown a heterogeneous response, which is unsurprising given the variability in recording location. 39% of the electrodes across 10 patients showed significant population activity that appears to encode for prediction error in the β and γ bands; i.e., the neural activity changed when the subject did not receive an expected reward at the appropriate time (See Fig 1B for example). Additionally, we see approximately 23% of electrodes across 6 patients show a significant response solely for when the reward was administered. These results are in line with the aforementioned animal studies, showing that STN activity is related to either reward stimuli and or the expectation of reward stimuli. Future studies will be done to link these results to the reward system as a whole using precise position data of the recording electrodes.



Disclosures: **J.R. Flynn:** None. **H.Z. Shouval:** None. **A. Fenoy:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant 5T32NS047987-10

Title: General and specific modulation of reward identity representations in human OFC after satiety

Authors: *J. D. HOWARD, T. KAHNT;
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Abstract: A hallmark of goal-directed behavior is the ability to flexibly pursue specific goals in the context of general motivational changes. For instance, finding both food and shelter is important, but just because we find food does not mean we should abandon the search for shelter. The same applies to food choices, where in a sated state, cues related to non-sated food items often continue to facilitate consumptive behavior. The implication is that even when sated, the brain maintains specific representations of non-sated outcome expectancies. We have previously shown that human orbitofrontal cortex represents the identity of expected food odor rewards. However, the mechanisms by which these representations are updated after satiety to reflect changes in expected reward value are not known. Here we combined functional magnetic resonance imaging (fMRI) with appetizing food odor stimuli and a sensory-specific satiety paradigm to address this question. Human subjects (N=17) first learned to associate visual symbols with two distinct food odors. Subjects then performed a task in which they made choices among these symbols in order to receive low-intensity (i.e. low value) or high-intensity (i.e. high value) versions of the odors while undergoing fMRI. Scanning was conducted first while participants were hungry, and then immediately after they had eaten a meal corresponding to one of the odors to satiety. In the hungry state, subjects reliably chose symbols associated with the high-intensity version of both odors. After eating, although ratings of hunger and desire to eat were diminished, subjects continued to choose the high-intensity version of the non-sated odor, while choices related to the sated odor were shifted towards the low-intensity. Multivoxel pattern analysis of the fMRI data revealed that representations of both odor identities in medial orbitofrontal cortex (mOFC) were completely abolished by satiety, while in lateral orbitofrontal cortex (lOFC) representations of the non-sated odor persisted in the full state. Moreover, we observed uniform satiety-related changes in outcome value in the ventral striatum (VS) and the ventromedial prefrontal cortex. These findings were mirrored by general satiety-related changes in connectivity between mOFC and VS, while coupling between lOFC and VS remained significant after the meal only for the non-sated odor. Taken together, these results suggest that

VS integrates information related to general changes in motivational state via mOFC, and identity-specific changes in value via lOFC, providing evidence for a novel mechanism by which separable regions of the OFC exert differential control over feeding behavior.

Disclosures: J.D. Howard: None. **T. Kahnt:** None.

Poster

645. Reward Learning in Humans

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Program#/Poster#: 645.17/LLL41

Topic: H.02. Human Cognition and Behavior

Support: NIH NS092079

Title: Reinforcement learning is not directly modulated by sensory prediction errors

Authors: *D. E. PARVIN[1], M. J. BOGGESS[1], S. D. MCDOUGLE[2], J. A. TAYLOR[2], R. B. IVRY[1];
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Abstract: Spilling a cup of hot coffee, at best, represents a failure to obtain an anticipated reward, and in some instances painful punishment. Obviously, it is not the coffee's fault but rather a failure of movement execution. Although this credit assignment appears intuitive, current decision-making models lack a mechanistic account of this process.
Recently, we explored this problem by employing a reaching version of a two-armed bandit task in which the failure to obtain an expected reward could result from motor execution errors (McDougle et al 2016). In this version of the task, participants showed a diminished risk-averse bias compared to that observed in a standard keyboard pressing version of the task, in which failed outcomes are attributed to malfunction of the bandit and not execution errors. Furthermore, we found that individuals with cerebellar degeneration failed to modulate their behavior between the two conditions. These results were consistent with a reinforcement-learning model in which value updating was gated by cerebellar-dependent sensory prediction errors (SPEs). The SPEs served as a signal to attenuate changes in value that normally occur following (negative) reward prediction errors.
Here, we tested this hypothesis by manipulating SPEs, independent of other possible confounding variables. In the original study, participants received movement feedback in the form of a cursor presented at the endpoint position of the movement. Unbeknownst to the participant, the experimenter controlled the cursor position, introducing small perturbations to control outcomes on some trials. Thus, movement feedback was veridical on some trials whereas

fake on other trials. This type of feedback may have induced a sense of control over the outcome in participants and it also prevented us from observing aftereffects, a hallmark of adaptation. To control for this in the current study, execution errors were signalled by the appearance of a cursor at an invariant midpoint location between the two targets. While participants are aware that the cursor in these displays does not indicate their hand position, a cerebellar-dependent adaptation system has been shown to treat this signal as an SPE and continues to adapt movement. Despite clear signatures of this adaptation, there was no effect on participants' choice behavior compared to a condition in which the visual feedback was absent. In both conditions, we observed the traditional risk averse bias. These results suggest that SPE signals do not directly solve the credit assignment problem, rather they may serve as a signal for the sense of competence in one's ability to produce actions that will achieve desired outcomes.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: Wellcome Trust Grant SJ1102

Title: Delays and dopamine at different times during reinforcement learning in Parkinson's disease patients

Authors: *J. P. GROGAN[1], D. TSIVOS[2], B. E. KNIGHT[3,4], R. BOGACZ[5], A. WHONE[4], E. J. COULTHARD[1,4];
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Abstract: Dopamine has been heavily implicated in reinforcement learning, however, recent work has begun looking at the role of dopamine in the expression of reinforcement learning, via consolidation, retrieval or exploration/exploitation mechanisms. We tested 18 Parkinson's disease (PD) patients and healthy age-matched participants (HPP) on a modified version of the Probabilistic Selection Task (PST), which tests to what extent participants learn from positive vs. negative feedback. In this task the participants were presented with pairs of cards and had to

learn which card to choose. One of the cards (A) was associated mainly with positive feedback, while another card (B) was associated predominantly with negative feedback. Thus the fraction of subsequent testing trials in which participants choose A and avoid B measures the tendency for learning from positive and negative feedback, respectively. Participants were tested with and without a 24 hour delay between learning and testing. Patients were ON or OFF their dopaminergic medication for learning, and in the 24 hours delay condition were also ON or OFF medication for the 24 hour test. We also tested PD patients on the canonical version of the PST. We looked at the different amounts of choose-A and avoid-B behaviour caused by the different medication states and delays. We found that after the 24 hour delay, PD patients and HPP showed a significant bias to avoid-B over choose-A, which was not affected by medication state at either time of learning, or time of testing. However, when patients were tested immediately after learning there was no such bias. This suggests that the information learned during reinforcement learning is not static, but is affected by delays between testing, and that this may affect the balance between positive and negative reinforcement learning. Computational reinforcement learning models with differential decays for the positively and negatively learned information were explored and fit to the data, and show that decay/consolidation mechanisms can account for the data.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: DGE-114747

R01-031310

Title: Striatum represents bayesian surprise, not reward prediction error, in rule learning.

Authors: *I. C. BALLARD[1], S. M. MCCLURE[2];
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Abstract: Objective: Humans naturally group the world into coherent categories defined by membership rules. While it is possible to learn these rules using stimulus-response associations, humans also have the ability to explicitly reason about rules. We sought to determine whether the

striatum, which reliably shows activation that scales with reward prediction error and is thought to be a part of the brain's reinforcement learning system, would also show prediction error responses in a task where people learn explicit rules.
Methods: We acquired functional magnetic resonance imaging data from subjects while they learned the category labels of stimuli with deterministic feedback. The stimuli had three dimension (shape, color, texture), and each dimension had two possible feature values. Subjects learned rules in blocks ranging from simple (e.g., blue stimuli are category 1) to complex (e.g., blue and square or checkered are category 1). We fit a series of reinforcement learning models that learns rules implicitly as well as a Bayesian rational rules model that reasons explicitly over rules.
Results: We exploit a difference in the learning signals between reinforcement learning models (reward prediction error) and Bayesian updating (surprise). Reward prediction error is a signed learning signal that is larger for positive than negative feedback. In contrast, surprise in our task was generally larger for negative feedback. In the neural data, we find greater BOLD signal responses to negative relative to positive feedback in both the striatum and ventral tegmental area. This pattern of responses is inconsistent with reward prediction error signaling. Further, we find that activation in the striatum scales parametrically with the Bayesian surprise signal. We propose that the striatum updates stimulus and action values, rather than representing learning signals per se. Although value updating and prediction error are perfectly correlated in reinforcement learning models, rule updating and surprise are not. We demonstrate that the striatum and caudal inferior frontal sulcus (cIFS) activation varies parametrically with rule updating and exhibits functional connectivity during feedback.
Conclusions: We conclude that the striatal feedback response does not represent prediction error as part of a reinforcement learning algorithm. Rather, its response scales with the relevant learning signal underlying learning because it is involved in updating a model of stimulus-response demands of the environment. We conclude that the striatum, in cooperation with the cIFS, is specifically involved in updating the values assigned to rules, rather than representation of error.

Disclosures: I.C. Ballard: None. **S.M. McClure:** None.

Poster

645. Reward Learning in Humans

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Topic: H.02. Human Cognition and Behavior

Support: Wellcome Trust Grant WT104765MA

James S McDonnell Foundation JSMF220020372

Title: Retrospective choice codes for causal learning

Authors: *E. D. BOORMAN[1], Z. KURTH-NELSON[3], L. RONDOT[3], M. GARVERT[3], R. J. DOLAN[3], T. E. BEHRENS[2];
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Abstract: Introduction
Humans have a remarkable capacity to flexibly pursue their goals in changeable environments. Such adaptive behavior requires the appropriate attribution of particular outcomes to particular past choices based on knowledge of the environment's causal structure. Here, we couple computational modeling of learning with multivariate 'representational' analysis approaches to reveal how particular reward identities are associated with their antecedent causal choices.
Methods
We scanned 20 healthy subjects with functional MRI while they performed a decision task. In condition 1 subject choices were resolved after a brief delay. In condition 2, comprising separate blocks of trials, choices were not resolved until subjects had witnessed the outcome of their *previous* choice and made a *second* decision. This manipulation meant the relevant causal choice was the most recent choice in condition 1 and the previous choice in condition 2. Preprocessing and univariate FMRI analyses were carried out using SPM12 and multivariate analyses using a nonlinear support vector machine implemented in Libsvm and Matlab 2014a.
Results
Behavior: Multiple linear regression analysis revealed that in condition 1 subjects appropriately credited outcomes to the *most recent* choice, ($choice_{t-1}Xoutcome_{t-1}$: $t(19)=9.07$, $p<0.0001$) but not the previous choice ($choice_{t-2}Xoutcome_{t-1}$: $t(19)=1.13$, $p>0.1$). Conversely, in condition 2 subjects instead appropriately credited the *previous* choice ($choice_{t-2}Xoutcome_{t-1}$: $t(19)=3.177$, $p=0.0025$) but not consistently the most recent choice ($choice_{t-1}Xoutcome_{t-1}$: $t(19)=-1.60$, $p=0.06$). This indicates subjects flexibly assigned credit to the appropriate causal choice based on the task rules.
FMRI: We tested whether the relevant past choice's identity elicited a characteristic activity pattern across voxels when outcomes were later revealed. In condition 1 the *most recent* choice could be decoded at the time of outcome delivery in inferior occipitotemporal, perirhinal and lateral orbitofrontal cortex ($t(19)>3.6$, $p<0.001$ uncorrected). In condition 2 we found a similar network for the *previous* causal choice's identity, albeit at a reduced threshold ($t(19)>2.57$, $p<0.01$), but not for the most recent choice ($t(19)<1.2$, $p>0.1$), which could instead be decoded from dorsolateral prefrontal cortex ($t(19)>2.57$, $p<0.01$).
Conclusions
Our findings provide novel evidence that past stimulus choices are coded in inferior occipitotemporal and orbitofrontal cortex when outcomes are subsequently delivered. This suggests retrospective choice codes provide a mechanism by which the brain attributes outcomes to their likely causes.

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Poster

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NSERC Michael Smith Foreign Study Supplements

Title: Updating impairments after right brain damage are due to inefficient exploration of hypothesis space.

Authors: *A. L. FILIPOWICZ[1], J. DANCKERT[1], E. KOECHLIN[2], P. DOMENECH[3], B. ANDERSON[1];
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Abstract: Recent research has demonstrated that patients with right brain damage (RBD) have difficulty updating their beliefs in response to environmental change. However, little is known about the functional basis of the impairment. The current study tests two potential explanations: a) RBD patients do not detect changes in their environment and therefore persist acting on old beliefs, or b) RBD patients detect changes, but fail to identify a new effective strategy due to an impaired ability to explore the hypothesis space. We administered an adaptive learning task to RBD and left brain damage patients (LBD). Participants had to learn rules about button-stimuli associations through trial and error. At unpredictable points throughout the task these rules changed (i.e., every 33-48 trials). On each trial, participants received noisy feedback (90% congruent) indicating whether or not they had pressed the correct button. We used a computational model of adaptive learning (the PROBE model) to partition the effect of change on exploratory behaviour. We found that RBD patients had more difficulty adapting to rule changes than LBD patients. These challenges, however, were not due to difficulties detecting rule switch; following a rule switch, RBD patients made changes to their responses, and persisted with old rules at a similar rate to LBD patients. Critically, RBD patients tended to detect *more* changes than were actually present. That is, RBD patients engaged in frequent and largely unguided explorations of potential new rules. We suggest that these difficulties stem from problems integrating feedback information such that RBD patients are more susceptible to

incongruent feedback. Our results suggest that the right hemisphere is specialized for the exploration of alternative options in adaptive learning tasks, and that exploratory failures contribute to the updating deficits seen in RBD patients.

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Poster

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Templeton Foundation Grant 21569

Title: Decoded fMRI neurofeedback can induce bidirectional behavioral changes within single participants

Authors: *A. CORTESE[1,2,3], K. AMANO[3], A. KOIZUMI[1,3], H. LAU[4,5], M. KAWATO[1,2]; [1]Cognitive Mechanisms Laboratories, Dep. of Decoded Neurofeedback, ATR Inst. Intl., Kyoto, Japan; [2]Information Sci., Nara Inst. of Sci. and Technol., Nara, Japan; [3]Ctr. for Information and Neural Networks, Osaka, Japan; [4]Dep. of Psychology, [5]Brain Res. Inst., UCLA, Los Angeles, CA

Abstract: Studies using real-time functional magnetic resonance imaging (rt-fMRI) have recently incorporated the decoding approach, allowing for fMRI to be used as a tool to manipulate fine-grained neural activity. Because of the tremendous potential for clinical applications, certain questions regarding decoded neurofeedback (DecNef) must be addressed. Neurofeedback effects have been previously shown to last for months, but the short- to mid-term dynamics are currently not known. Specifically, can the same participants learn to induce neural patterns towards two opposite directions in different sessions? This leads to a further question, whether learning to reverse a neural pattern may be less effective after training to induce it in a previous session. Here we employed a within-participants design, with participants undergoing

DecNef training sequentially in opposite directions (up or down regulation of confidence judgements in a perceptual decision task), with the order counterbalanced across participants. For each direction, participants underwent two days of DecNef training, with behavioral tests before and after the fMRI scanning sessions (pre- and post-tests). A one-week interval separated the opposing DecNef trainings. Behavioral results indicated that the magnitude of neurofeedback effect was strongly influenced by the order and direction of neurofeedback training. We therefore applied nonlinear mathematical modeling to decompose the behavioral effect into four main consequences of DecNef: main effect of change in behavior, strength of down-regulation effect relative to up-regulation, maintenance of learning over sessions, and anterograde learning interference. Modeling results revealed that bidirectional brain manipulation successfully led to bidirectional behavioral changes in different sessions. Furthermore, up-regulation effect was more sizable, and such effect was largely preserved even after an interval of one week. Lastly, there existed a strong anterograde interference onto the next DecNef session by the first week DecNef; the consequence was a stronger effect of neurofeedback in the first week as compared to the second week. These results suggest reinforcement learning characteristics of DecNef, and provide important constraints on its application to basic neuroscience, occupational and sports trainings, and therapies.

Disclosures: **A. Cortese:** None. **K. Amano:** None. **A. Koizumi:** None. **H. Lau:** None. **M. Kawato:** None.

Poster

645. Reward Learning in Humans

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F32-MH102009

Title: Dissociable explanations for uncertainty driven changes in neural representation across the brain

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Abstract: Neural networks in rodent medial prefrontal cortex occasionally go through rapid changes in neural firing patterns. Such “network resets” tend to occur during periods of uncertainty during which animals are rapidly shifting between different behavioral regimes [1]. Currently it is unknown what the populations of neurons that participate in such phenomenon are representing. One potential explanation is that such populations represent the current behavioral regime and that rapid transitions in this representation reflect changes of mind with regard to a behavioral policy. An alternative explanation for this phenomenon is that the networks provide a fluctuating context to which newly arriving information is bound. Within this framework, gradual transitions in network activity could allow partial pooling of information collected within a stable context, whereas rapid network resets could allow information collected in one context to be effectively partitioned from that collected in the next. The critical difference between these two explanations is that the former involves a population representation that is capable of returning to a previously encountered behavioral regime whereas the latter explanation predicts a population representation that moves further and further from previous contexts, even when those contexts involved similar stimulus action mappings. Thus, testing these two hypotheses requires observing uncertainty driven network resets in the context of a task where behavioral regimes are occasionally re-encountered. Here we do this by examining the fluctuations in multi-voxel patterns of BOLD activity from human subjects making sequential inferences about the state of a partially observable and discontinuously changing variable [2]. We find that, within the context of this task, the vast majority of cortex contains representations that change more rapidly during periods of uncertainty. We find that, in some cortical areas, such as motor cortex, this phenomenon is indicative of discontinuous changes in the representation of a behavioral regime. In contrast, we identify other cortical regions, including orbitofrontal cortex, which are more consistent with the representation of a context that fluctuates more rapidly during periods of uncertainty. Finally, we show that several other cortical areas showing the basic phenomenon, such as DLPFC and ACC, are best described by an alternative explanation: encoding uncertainty itself.
1. Karlsson MP, Tervo DGR, Karpova AY**.** *Science* 2012, **338**:135-139.
2. McGuire JT, Nassar MR, Gold JI, Kable JW**.** *Neuron* 2014, **84**:870-881.

Disclosures: J.W. Kable: None. **M. Nassar:** None. **J. McGuire:** None.

Poster

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Title: The network architecture of value learning

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Abstract: Value guides behavior. With knowledge of stimulus values and action consequences, one is able to select its actions to maximize expected cumulative reward. Prior work has identified several brain structures critical for representing stimuli and their values. It remains unclear, however, how these structures interact with one another and with other regions of the brain to support the acquisition of value-related knowledge. Here we approach this problem from a network neuroscience perspective, examining patterns of BOLD functional connectivity across the whole brain and how they change as 20 human subjects learn the values of novel stimuli in a simple two-alternative forced choice task. The monetary values of 12 shapes were learned over the course of four consecutive days, through a feedback consisting either of the value of the shape selected (10 subjects) or the correctness of the selection (10 subjects). Our results show that the overall functional networks change continuously over the course of learning, and that these changes primarily involve regions of the visual, frontal, and anterior cingulate cortices. Using a cross-validation procedure, we show that connections between visual and frontal and between visual and cingulo-opercular modules are useful predictors of a subject's learning stage, and that connections between various modules and basal ganglia structures can significantly classify the type of feedback received by the subjects. Together, these results demonstrate that functional networks dynamically track behavioral improvements in a value judgment task, and that interactions between network communities are useful biomarkers of value learning.

Disclosures: **M.G. Mattar:** None. **S.L. Thompson-Schill:** None. **D.S. Bassett:** None.

Poster

645. Reward Learning in Humans

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Topic: H.02. Human Cognition and Behavior

Title: Sequential integration of task-related dimensional components during multi-dimensional reinforcement learning task

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Abstract: We often face multi-dimensional decision making situations, in which we gather incomplete pieces of information to deduce optimal decisions. Although a few studies have been performed on reward-based learning process in multi-dimensional environments, they focused only on the reduction of unnecessary dimensions (Niv et al., 2015), competitions, or combinations of dimensions (Hunt et al., 2014); no previous study has yet investigated how the brain integrates dimensions during learning trials. In this study, we investigated how the brain integrates information in multi-dimensional reinforcement learning task using computational model based on hidden markov model (HMM) and reinforcement learning (RL) and fMRI; we observed that our brain integrates pieces of information in a sequential manner and that the frontopolar cortex is responsible for the integration process.
23 subjects participated in the multi-dimensional behavioral task. Subjects were shown pictures with multiple features—shapes, color, and patterns—and asked to collect as many points (reward) as possible; participants were not informed of the rule and were to find optimal strategies to achieve their goals. During behavioral tasks, subjects' performance and the fMRI signals were simultaneously recorded.
To examine whether subjects learned by integrating multi-dimensional information sequentially or processing concurrently, we fitted behavior data into two separate computational models—one with both HMM and RL for sequential learning and the other with only RL for concurrent learning. The comparison between two models demonstrates that the participants integrated multi-dimensional information sequentially.
After confirming that information is integrated sequentially, we examined which part of the brain was related in our behavioral task from the fMRI data. We observed that the frontoparietal cortex encoded expected reward, and the ventromedial prefrontal cortex encoded prediction error, as previously reported (Lee et al., 2014). Also, the frontopolar cortex was observed to be correlated with transition probabilities in HMM, indicating that it is responsible for multi-dimension integrating process. Psycho-physiological interaction (PPI) analysis revealed the existence of causal connections between these three areas, suggesting that the frontopolar cortex works as a meta-controller and integrator of value and error signals.
Overall, our result explains how our brain integrates multi-dimensional components sequentially during reinforcement learning. Our study, therefore, suggests how human effectively processes new information when the dimension is extended.

Disclosures: O. Choung: None. **Y. Jeong:** None.

Poster

645. Reward Learning in Humans

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Topic: H.02. Human Cognition and Behavior

Support: DFG, EXC 1086

Title: Correlates of robot-error observation in the human EEG

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Abstract: Brain-controlled robots are a new type of assistive device for severely impaired persons. Information about the correctness of robot performance might provide a teaching signal for adaptive control algorithms and thus help reducing errors. To reach this goal, an evaluation of the correctness of the robot's action must be inferred from the brain activity of the robot user.Studies about the neural correlates during observation of robotic action are rare. Here, we present findings from two novel experiments in which subjects watched different types of robots conducting everyday-actions (i.e. pouring liquid and picking up objects). In both scenarios, actions were either performed correctly or incorrectly (Fig. 1 a-c). We recorded 128-channel high-resolution EEG during the observation tasks to train rLDA- and filterbank-CSP classifiers on varying time-intervals and frequency-components of the multivariate signal. These classifiers should either decode for the correctness of the observed action, or for the type of the observed robot (humanoid/non-humanoid). It was possible to decode both the correctness, at least in one of the investigated error scenarios (spilling of liquid), as well as the robot type significantly above chance level with decoding accuracies up to around 70% (see Fig. 1 d-f). Further on, our results indicate an effect of the frequency components used for decoding. We conclude that non-invasive recordings of brain activity elicited when observing robots can indeed provide decodable information about the correctness of the robot’s action, and also about the type of observed robot. Hence, brain signals related to robot-error observation may improve the performance of future brain-controlled devices and might reveal valuable insights into the neural bases of human-robot interaction in general.

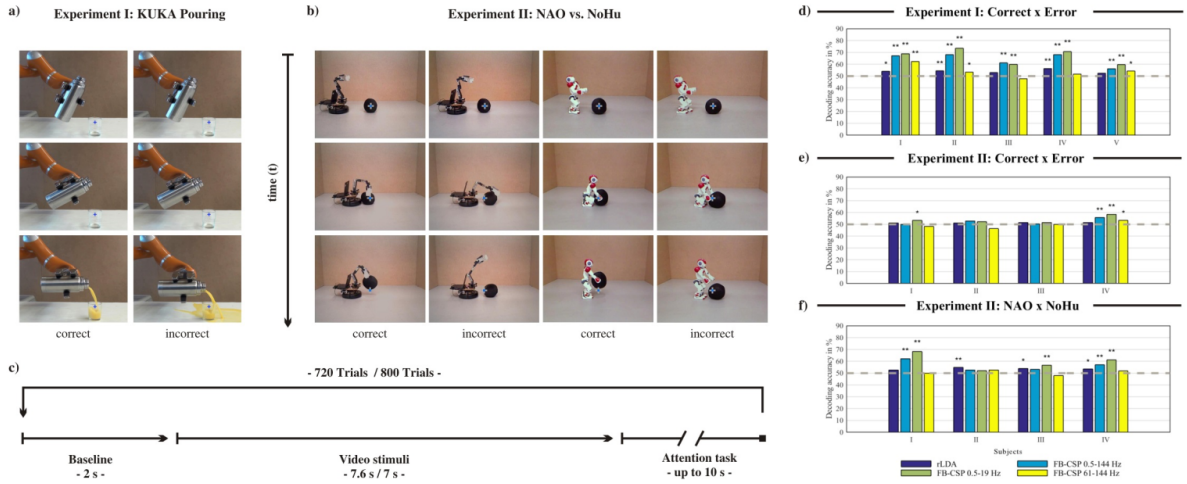

Figure 1. Experimental paradigms and decoding results: video stimuli in exp. I (a) and exp. II (b), and schematic procedure of the observation tasks (c). Best filterbank-CSP decoding results for exp. I (d), error condition of exp. II (e) and robot condition of exp. II (f). Significance is indicated by asterisks: * $p < 0.05$, ** $p < 0.01$.

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Poster

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Title: Prediction-error signals in the substantia nigra and the dorsal striatum for behaviorally relevant Pavlovian learning relate to performance at instrumental learning

Authors: *J. T. COLAS[1], W. M. PAULI[2], T. LARSEN[2,3], J. M. TYSZKA[2], J. P. O'DOHERTY[1,2];
[1]Computation and Neural Systems Program, [2]Div. of the Humanities and Social Sci., Caltech, Pasadena, CA; [3]Ctr. for Mind/Brain Sci., Univ. of Trento, Trento, Italy

Abstract: In natural settings, Pavlovian (or classical) conditioning and instrumental (or operant) conditioning often occur concurrently. Neural subsystems specialized for either type of learning thus often could benefit from exchanging information. Typical approaches to studying the interactions between these two systems entail presenting previously learned Pavlovian cues during instrumental performance and exploring the interactive effects on behavior (i.e., Pavlovian-instrumental transfer). Here, we were more concerned with the extent to which knowledge about the environment acquired via Pavlovian learning (i.e., conditioned reinforcement) could influence instrumental learning when the outcomes of those instrumental actions are learned about in a Pavlovian manner, and we set out to delineate the neural mechanisms underlying such effects.
For this study, human subjects performed a multi-stage learning task that required both Pavlovian and instrumental learning in parallel. High-resolution functional magnetic-resonance imaging (fMRI) data with 1.5-mm isotropic voxels were acquired using protocols optimized for the midbrain. Reinforcement-learning models accounted for subjects' behavior and were applied to model-based analyses of the fMRI data. Pavlovian states were interleaved with and related to instrumental states by design. Prediction-error signals for these Pavlovian states were identified within the dopaminergic midbrain--specifically, the substantia nigra and the ventral tegmental area--as well as in the ventral striatum, a region that has previously been implicated in Pavlovian learning. Notably, such Pavlovian learning signals were also identified within the dorsal striatum, a region that has previously been argued to have roles specific to instrumental learning. Furthermore, the magnitudes of Pavlovian prediction-error signals in the substantia nigra and the dorsal striatum were correlated with how optimally subjects performed the instrumental component of the task.
The present findings suggest that acquiring conditioned reinforcement through Pavlovian learning may depend on neural circuits hitherto implicated in instrumental conditioning--namely, in the dorsal striatum and the parts of the substantia nigra that project to the dorsal striatum. By linking the dorsal striatum to learning about conditioned reinforcers that ultimately could be used to drive instrumental actions, the present results broaden our understanding of the functions of this area in reward-related learning.

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Poster

646. Human Cognition: Attention II

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Topic: H.02. Human Cognition and Behavior

Support: ITN- 2013-606901

Title: Does the salience network's functional connectivity relate to individual variability in processing speed in elderly subjects?

Authors: *A. RUIZ-RIZZO[1], J. NEITZEL[2], N. NAPIORKOWSKI[1], H. J. MÜLLER[1], C. SORG[2], K. FINKE[1];
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Abstract: Visual processing speed is the rate of visual information uptake per time unit. The salience network (SN) covers bilateral frontal gyrus areas assumed to be relevant, e.g., for processing speed. While both processing speed and intrinsic functional connectivity (iFC) of the SN (SN-iFC) are reduced in healthy aging, it is not known whether and how their individual variability is related. Here, we aimed to describe this relation in a group of older (60-80 years; mean = 71; n = 29; 11 females) and, for reference, a group of younger (18-35 years, mean = 25.9; n = 26; 14 females) adults. All participants underwent resting-state functional magnetic resonance imaging for 12.5 min, with eyes closed, in a 3T scanner. In a different session, processing speed was assessed using a Theory of Visual Attention (TVA)-based whole-report task. Here, participants were presented with letter arrays for brief, variable, and individually pre-adjusted exposure durations and had to report all letters they were fairly certain they had seen. Parameter processing speed was estimated, via computational modelling of report accuracy changes with increasing exposure durations. Preprocessed functional images of all participants were used for a group independent component analysis with 20 dimensions. The SN was visually identified and further confirmed by multiple regression of a SN template. To examine the correlation between SN-iFC and processing speed values, we performed multiple regression analysis of both variables, separately for each age group. In the older compared to the younger group, we found significantly lower visual processing speed and lower average SN-iFC. Significant group differences were found in IFC in left superior frontal and middle frontal gyrus, left anterior and right middle cingulate cortex, and right angular gyrus. Within each group, we found significant correlations between individual visual processing speed values and SN-iFC in distinct frontal clusters, i.e. a left inferior cluster in the older and a right middle frontal cluster in the younger group. Our results suggest that, within the SN, frontal iFC is associated with visual processing speed across different age levels, but that the most critical frontal area within the SN might change with age.

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Poster

646. Human Cognition: Attention II

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Program#/Poster#: 646.02/LLL53

Topic: H.02. Human Cognition and Behavior

Title: The impact of input modality on cognitive training task performance in a large online population

Authors: S. MACLEOD, *A. KALUSZKA;
Lumos Labs, Inc., San Francisco, CA

Abstract: Previous research has shown that varying psychophysical task parameters can have significant effects on performance. However, this research did not account for different input methods and their effects on cognitive task performance. We hypothesized that altering the input modalities in cognitive training tasks would affect participant performance and training strategies.
Through a wide-scale study of participants undergoing cognitive training through an online system (Lumosity), which includes millions of users, we investigated the result of input method differences between computer-based and mobile-based training. We measured performance in several different cognitive training tasks, including selective and divided attention and working memory tasks.
Performance metrics such as reaction time, error rates, and memory capacity were recorded and compared across groups completing either computer-based or mobile-based training. We observed several significant differences in behavior, such as faster response times and lower accuracy in mobile-based attention tasks compared to computer-based tasks. This study suggests that input modality is an important consideration when designing cognitive training tasks and interpreting their results.

Disclosures: **S. MacLeod:** A. Employment/Salary (full or part-time): Lumos Labs, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs, Inc. **A. Kaluszka:** A. Employment/Salary (full or part-time): Lumos Labs, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs, Inc..

Poster

646. Human Cognition: Attention II

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Topic: H.02. Human Cognition and Behavior

Support: DFG, SFB 779, TPA2 and TPA3

Title: Attentional modulation of neural oscillations in the human spinal cord

Authors: *M.-P. STENNER[1], T. ZAEHLE[2], E. AZAÑÓN[4], H.-J. HEINZE[2], A. SCHOENFELD[5], L. BUENTJEN[3];
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Abstract: Neural oscillations in sensory cortex, recorded via scalp electroencephalography (EEG), are modulated by attention and expectation. In particular, cortical alpha- and beta-oscillations are suppressed in anticipation of attended stimuli, and this pre-stimulus suppression influences perception. We demonstrate a similar top-down oscillatory modulation at the level of the human spinal cord. We recorded local field potentials (LFPs) from epidural spinal electrodes implanted for pain relief in patients with different pain syndromes, simultaneous to scalp EEG, during an intermodal, somatosensory vs. visual, attentional cueing paradigm. We found a stimulus-induced decrease in the power of alpha- and beta-oscillations in spinal LFPs, similar to stimulus-induced responses in the cortical EEG. Importantly, the power of alpha- and low beta-oscillations in the spinal cord was already suppressed prior to stimulus onset, evidence of an anticipatory, top-down regulation at the level of the spine. Attention to somatosensory stimuli resulted in diminished, rather than enhanced, pre-stimulus alpha- and beta-suppression, contrary to its well-known effects at the cortical level. Taken together, first results of this ongoing study support the idea of a top-down regulation of somatosensory processing via changes in alpha- and beta-oscillations as early as the spinal cord.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH R01 NS055829

Title: Intracranial cortical event-related potentials and alpha wave gating of visual consciousness.

Authors: *S. I. KRONEMER[1,2], W. R. XIAO[1,2], L. GOBER[1], R. E. SMITH[1], S. M. A. WAFA[1], A. RAJA[1], E. MORSE[1], R. E. WATSKY[1], C. L. HORIEN[1], E. SABERSKI[1], O. MORGAN[1], G. J. TOULOUMES[1], W. C. CHEN[1], D. D. SPENCER[3], J. L. GERRARD[3], H. BLUMENFELD[1,3,2];
[1]Neurol., [2]Neurosci., [3]Neurosurg., Yale Univ., New Haven, CT

Abstract: Primary questions in the study of consciousness are (1) what is the succession of neural activity linked with conscious events, and (2) how are otherwise unconscious stimuli recruited to perception? The current investigation supports the role of alpha oscillations in gating conscious perception and corroborates the neural activity progression defined by established models of visual consciousness. Nine participants with intractable epilepsy undergoing an intracranial EEG implantation (100-300 subdural intracranial electrodes; sampling rate = 1024 Hz) were recruited from the Yale Epilepsy Surgery Program. A visual threshold perception task was administered requiring participants to indicate their awareness of a face stimulus appearing on a dynamic background. Stimuli were calibrated to that participant's 50% threshold of contrast detection, confirmed by response accuracy. Electrodes located in the primary visual cortex revealed similar intracranial event-related potentials (ERPs) activity between perceived and not perceived trials during early post-stimulus onset phases (<200 ms). Diverging ERPs were observed between perceived and not perceived trials during intermediate and late post-stimulus onset phases (>200 ms). Deviations were particularly true for higher visual processing and other association cortical areas, including the fusiform face area, fronto-parietal cognitive and medial temporal memory networks. Moreover, alpha oscillations tended to be phase locked within both consciously perceived and not perceived trials, yet phase shifted relative to these trial types within subjects. Accordingly, the temporal relationship between alpha phase and the stimulus onset may gate conscious events. In summary, these results exhibit unique activity patterns for higher order brain circuits for consciously perceived versus not perceived stimuli. Furthermore, the alpha phase data suggests that pre-stimulus activity is involved in gating conscious perception. Our ERP and alpha findings predict global synaptic brain activity that may be essential for defining what stimuli become conscious and how this information is processed.

Future investigations will focus on the role of subcortical activity in conscious perception and its relationship to the temporal progression and regional specific activity for conscious stimuli as revealed in the current study.

Disclosures: **S.I. Kronemer:** None. **W.R. Xiao:** None. **L. Gober:** None. **R.E. Smith:** None. **S.M.A. Wafa:** None. **A. Raja:** None. **E. Morse:** None. **R.E. Watsky:** None. **C.L. Horien:** None. **E. Saberski:** None. **O. Morgan:** None. **G.J. Touloumes:** None. **W.C. Chen:** None. **D.D. Spencer:** None. **J.L. Gerrard:** None. **H. Blumenfeld:** None.

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01-DA013165

Title: Template specificity in loss avoidance value driven attentional capture

Authors: *M. DIBARTOLO[1], B. A. ANDERSON[1], S. M. COURTNEY[1,2,3];
[1]Psychological & Brain Sci., [2]Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD; [3]F.M. Kirby Res. Ctr. for Functional Brain Imaging, Kennedy Krieger Inst., Baltimore, MD

Abstract: Cues previously associated with reward persistently capture attention, even when rendered task-irrelevant and perceptually non-salient. This phenomenon, referred to as value-driven attentional capture (VDAC), has been studied in the context of reward, but the role of active loss avoidance in shaping attentional biases remains unexplored.
To explore the ability of negative reinforcement to support VDAC, human participants completed two behavioral tasks over the course of a single session. In an initial training phase, participants learned to associate one color with low value (3¢) and another color with high value (15¢) during visual search for a color-defined target. Correct responses were associated with the avoidance of a corresponding loss from a compensation bank. The shape of the target and non-target stimuli were only circles for 32 participants (10 male, age 21.63 $\pm$ 3.9) and only diamonds for 19 participants (10 male, mean age 20.44 $\pm$ 2.2). Following the first task, all participants completed a second visual search task during which they identified a unique shape target among colored distractors. Half of these trials included a previously value-associated color distractor, which either was or was not the shape of the trained stimuli (equally-often). Trials that included distractors in the same color and shape as the value-associated stimuli during training were considered template matches. Reaction times were compared across value conditions, separately

for distractor shape template match and non-match conditions.
Response times (RTs) differed significantly among the three test phase value conditions: distractor absent (644 $\pm$ 74 ms), low value distractor (649 $\pm$ 80 ms) and high value distractor (655 $\pm$ 76 ms) [$F(2,100) = 4.7$, $p < .05$]. High-value distractors slowed RT relative to distractor absent trials [$t(50) = 3.212$, $p < 0.01$]. When comparing across trial types, a repeated-measures ANOVA revealed an interaction between training shape and the template match of the distractors [$F(1,49) = 112.683$, $p < .001$]. The high-value distractors slowed RT relative to distractor absent trials specifically in template matching [$t(49) = 4.228$, $p < .001$], but not non-matching [$t(49) = .643$, $p = .523$] conditions.
The results indicate that, as with monetary gains, stimuli associated with loss avoidance are able to capture attention. However, unlike with monetary gains, loss avoidance VDAC is only significant when the distractor in the test phase is an exact match to both the color and shape of the stimulus for which the value association was learned during the training phase.

Disclosures: **M. Dibartolo:** None. **B.A. Anderson:** None. **S.M. Courtney:** None.

Poster

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Program#/Poster#: 646.06/LLL57

Topic: H.02. Human Cognition and Behavior

Title: Costs and benefits of tRNS-induced improvements in performance during an unattended feature task

Authors: *S. TYLER[1], F. CONTÒ[2], L. BATTELLI[3,4];
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Abstract: Attention helps selectively sensitize the brain towards important visual information. However, repetitive exposure to either below threshold or unattended stimuli can still sensitize the visual system to those features (Watanabe 2001). In our study, we investigate whether transcranial random noise stimulation (tRNS) facilitates this sensitization during an unattended feature paradigm, and whether the presence of a competing task interferes with this process. Fifty-two participants were equally divided into a “Training” or “No Training” group. Both groups completed an orientation discrimination task on Day 1 and Day 5. The task comprised of a pair of temporally offset flickering discs (black and white), with one cycle briefly presented as

a pair of oriented Gabors. Subjects were asked to judge if the orientation of the Gabors were the same. On Days 2-4, subjects in the "Training" group were presented with the same stimuli, but instead asked to attend to the temporal order of the oriented Gabor discs. The "No Training" group had no task.
These groups were further divided into one of four tRNS groups: bilateral Parietal, bilateral hMT+, bilateral Sham, and No Stim condition. Subjects undergoing active (Parietal and hMT+) or Sham stimulation were stimulated during the temporal order task on Days 2-4 for 25 minutes each day.
Parietal stimulation in the "No Training" group significantly *increased* behavioral accuracy for the orientation discrimination task on Day 5 versus Day 1. All other tRNS conditions showed no net improvement over the week. Conversely, parietal stimulation in the "Training" group caused *decreased* accuracy on the orientation task on Day 5 versus Day 1. This trend was not seen in any other "Training" stimulation groups.
These results are the first to show that tRNS over parietal cortex can selectively induce task-specific excitation for the attended task (the temporal order task) while suppressing performance on a task irrelevant perceptual task (the orientation task). tRNS can act as an exogenous boost to attentional arousal, allowing subjects to better focus on a continuous selective attention task (Mauri et al, 2015). For subjects in the "No-training" condition, this boosted state may linger into Day 5, producing a heightened state of cognitive arousal allowing subjects to better ignore competing temporal order information. However, for the "Training" group, specific parietal cortical networks involved in temporal order processing were boosted via tRNS, and that effect interfered with behavioral performance on the orientation task.

Disclosures: **S. Tyler:** None. **F. Contò:** None. **L. Battelli:** None.

Poster

646. Human Cognition: Attention II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 646.07/LLL58

Topic: H.02. Human Cognition and Behavior

Title: Novel mobile video game and electroencephalography system to assess the neural correlates of attention

Authors: ***R. GIL-DA-COSTA**, R. FUNG, M. ZINNI, M. CASWELL, M. LOPES; Neuroverse, Inc., San Diego, CA

Abstract: In a society currently affected by a wide range of mental disorders that impose exponentially growing heavy penalties at both the individual and societal levels, there is a

concerning absence of usable and reliable systems to assess cognitive and neural function in large scale. Unlike other biosensors (i.e. cardiac, glucose, etc.), available solutions in the neural space are cumbersome, unreliable or both, and as such there is an urgent need for novel tools designed to capture, integrate and evaluate brain signals of interest in real-world monitoring settings. Here, we used Neuroverse's mobile forehead electroencephalographic (EEG) sensing system, with a software application for testing and analysis in mobile platforms (e.g. smartphones and tablets) to test the detection of well-known event-related brain potentials (ERP) correlated with attention (P300 and N2) in 25 healthy individuals during two visual oddball paradigm tasks: a) a more traditional serial visual presentation of natural images (where subjects were asked to keep a mental count of "target" images) and b) a novel action video game (where subjects were required to discriminate and selectively respond to "red" ants while simultaneously filtering and withholding a response to the more frequently presented "black" ants). Our results show statistically significant differences for brain responses to "target" versus "distractor" objects in both tasks, demonstrating the efficacy of this mobile system for efficient large scale use in the assessment of attentional neural responses. Moreover, additional advantages of the use of the video game versus more traditional testing paradigms, both in terms of additional measures and engagement levels, will be discussed. This study presents evidence of the outstanding efficacy of a novel user-friendly mobile system that can open new avenues for much needed large-scale applications ranging from clinical (i.e. neuropsychiatric deficit detection and evaluation, clinical outcome predictability and drug efficacy evaluation) to health prevention or education.

Disclosures: **R. Gil-Da-Costa:** A. Employment/Salary (full or part-time): Neuroverse, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuroverse, Inc. **R. Fung:** A. Employment/Salary (full or part-time): Neuroverse, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuroverse, Inc. **M. Zinni:** A. Employment/Salary (full or part-time): Neuroverse, Inc. **M. Caswell:** A. Employment/Salary (full or part-time): Neuroverse, Inc. **M. Lopes:** A. Employment/Salary (full or part-time): Neuroverse, Inc..

Poster

646. Human Cognition: Attention II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 646.08/LLL59

Topic: H.02. Human Cognition and Behavior

Support: Deutsche Forschungsgemeinschaft Grant Collaborative Research Center 889

Deutsche Forschungsgemeinschaft Grant Research Unit 1847

Title: Sustained spatial attention alone explains the bias in microsaccade direction

Authors: ***C. XUE**[1], A. CALAPAI[1], K. DANNHAEUSER[2], J. KRUMBIEGEL[2], S. TREUE[1,2]; [1]German Primate Ctr., Goettingen, Germany; [2]Fac. of Biol. and Psychology, Goettingen Univ., Goettingen, Germany

Abstract: Microsaccades are small eye movements while fixating. The distribution of their directions is biased towards an eccentric location indicated by an endogenous spatial cue ~300ms after the cue's presentation. Therefore, microsaccades could serve as an index for covert spatial attention. However, this use of post-cue microsaccades suffers from two major factors. First, visual spatial attention is usually entangled with saccade planning. A systematic investigation is needed, as to which extent saccade planning can influence microsaccade directions, confounding the attention-specific effect. Second, most studies of the post-cue microsaccade direction effect focused on a time window around 300ms after the onset of the endogenous cue (such as an arrow pointing at the required gaze location); and the bias reverses direction if the cue is exogenous (i.e. appears at the required location). These effects seem to indicate that the microsaccade direction bias is contingent on the cue, not necessarily reflecting a sustained attentional state. To resolve the first problem, we investigated microsaccades of human subjects while they performed a spatial attention guided match-to-sample task. The subjects had to covertly attend to a location while preparing a saccade either toward or away from the attended location. To resolve the second problem, we provided a verbal spatial cue before each experiment session, instead of before each trial, so as to exclude any cue contingent effects on micro saccades. Our data show a sustained bias of the directions of microsaccades toward the attended location, and not the saccade target. Despite the absence of cues during the trials, sustained attention alone still reliably produces the microsaccade direction effect. Overall, our findings demonstrate that sustained spatial attention, rather than saccade planning or the spatial cue per se, determines the direction bias in microsaccades. Our results also question the notion that spatial attention is functionally equivalent to a movement planning process.

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Poster

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Program#/Poster#: 646.09/LLL60

Topic: H.02. Human Cognition and Behavior

Support: NSERC Grant

Title: Investigating the relation between Stroop interference and attention capture using event-related potentials

Authors: A. J. LOWERY, R. D. WRIGHT, *J. J. MCDONALD;
Psychology, Simon Fraser Univ., Burnaby, BC, Canada

Abstract: People are faster to name the color in which a word is printed (e.g., blue) when the word itself is a congruent color word ("BLUE") than when it is an incongruent color word ("RED"). This well-known Stroop effect has been ascribed to the automaticity of word reading. That is, the Stroop words are read in an obligatory fashion (despite the fact that they are task-irrelevant), and the meanings of those words are extracted without necessarily attending to the words. However, some results suggest that the magnitude of Stroop interference depends on attention. For example, Kahneman and Chajczyk (1983) found that the Stroop effect is reduced by the concurrent presentation of a response-irrelevant stimulus, such as a string of identical letters (e.g., "XXX"). This reduction of Stroop interference was hypothesized to occur because the second stimulus interferes with attentional processing of the color word, implying further that the Stroop effect itself depends on the ability of the color word to capture attention. In the present study, we recorded event-related potentials (ERPs) to determine whether (i) task-irrelevant color words capture attention in the non-integrated variant of the Stroop task, and (ii) the magnitude of Stroop interference depends on the strength of attention capture. On each trial, a colored rectangle (red, green, or blue) was presented at fixation and was flanked by a color word ("RED", "GREEN", or "BLUE") on one side and a length-matched string of Xs on the other side of fixation. Participants were required to identify the color of the rectangle and to press a corresponding button as quickly and as accurately as possible. Consistent with previous findings, response times were slightly but significantly longer on incongruent trials than on congruent trials (18 ms difference). To determine whether the color word captured attention, we isolated lateralized ERP components associated with early attentional selection (N2pc) and later stimulus identification (SPCN). Color words were found to elicit both the N2pc and subsequent SPCN, demonstrating that the words were attended despite the fact that they were irrelevant. However, the amplitudes of these ERP components did not correlate with the magnitude of Stroop interference. These findings indicate that while the color words captured attention reflexively, such attention capture may not underlie the interference found in the Stroop task.

Disclosures: A.J. Lowery: None. **R.D. Wright:** None. **J.J. McDonald:** None.

Poster

646. Human Cognition: Attention II

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Program#/Poster#: 646.10/LLL61

Topic: H.02. Human Cognition and Behavior

Support: US-Israel Binational Science Foundation (2013400)

Title: Object-based attention acts upon degraded object representations in early visual cortex

Authors: *S. AL-JANABI[1], N. STROMMER-DAVIDOVICH[2], S. GABAY[2], A. S. GREENBERG[1];
[1]Dept. of Psychology, Uw-Milwaukee, Milwaukee, WI; [2]Univ. of Haifa, Haifa, Israel

Abstract: Previous evidence of object-based selection along the visual cortical hierarchy (V1 to V4) indicates that attention can gate visual (object) representations early in the processing stream. It is unclear, however, whether or not the strength of object percepts modulates the level along the visual cortical hierarchy at which object-based selection is evident. Here, we aimed to ascertain whether attention selects information 'early' or 'late' in the visual processing stream when object information is degraded. While acquiring fMRI data, we asked participants to identify a target preceded by a central arrow cue (60% valid) in the double-rectangle paradigm (Egly et al., 1994). Cues to objectness were strong (standard visible rectangles), reduced (occluded rectangles), or weak (illusory rectangles). Our behavioural results indicated that participants identified the target faster when it appeared on the spatially cued versus the non-cued object. This same-object advantage emerged irrespective of object strength, suggesting that both strong and weak object percepts can be prioritised for attentional selection. We independently localized retinotopically-specific regions of cortex corresponding to target locations to examine neural activation in both early and mid-level regions of the visual cortex hierarchy (V1-V3, and LOC). Consistent with our behavioral results, target-evoked BOLD activation in V1/V2 increased at locations on the cued object, but not the non-cued object; indicative of a neural same-object advantage in these regions. This effect did not differ as a function of object cue strength, and it was absent in both V3 and LOC. We, therefore, conclude that attention modulates weak object percepts at the earliest levels of the visual cortex hierarchy. This finding has implications for theories of attentional selection, supporting early selection accounts, which posit that attention serves to help bind low-level visual information into completed object percepts.

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Poster

646. Human Cognition: Attention II

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Topic: H.02. Human Cognition and Behavior

Support: Mind and Life Francisco J Varela Award

Title: Neurofeedback Informed Meditation Technique (NIMT) for modulating posterior cingulate cortex activity—proof-of concept for a novel mental-training paradigm with clinical applications

Authors: *J. F. SANTOYO[1,2], B. CULLEN[3], C. KERR[4];
[1]Providence, RI; [2]Neurosci., [3]Psychiavtry and Human Behavior, [4]Family Med., Brown Univ., Providence, RI

Abstract: I present initial evidence from two experiments for a method (NIMT) that aims to elucidate clinically-relevant causal relationships between neurophysiological and cognitive processes. Here, expert meditators use a neurofeedback display to employ a systematic procedure for discovering, formulating and refining meditation strategies (NIMTs) that modulate the chosen neurofeedback signal. NIMTs are then introduced to hypothesis-blind groups to assess the influence of the NIMT on the targeted neurophysiological signal and relevant behavioral measures. In our first experiment, we developed a NIMT for downregulating posterior cingulate cortex (PCC) activity by drawing on expert meditators' verbal descriptions of the experiential correlates of PCC activity reported previously (Garrison et al. Frontiers in Human Neurosci . 2013). We used fMRI to compare PCC activity and functional connectivity in participants with some meditation experience (n=10) during six minute blocks during which participants used either the PCC-NIMT or a control meditation (focused attention on the breath) and rest-blocks with the instructions to 'close your eyes, do not meditate and do nothing in particular'. BOLD activity in the PCC was significantly decreased during the PCC-NIMT in comparison to rest and the control meditation. In our second experiment, expert meditators (n=4) used EEG-based neurofeedback to develop NIMTs for upregulating somatosensory alpha-band (S1-alpha) power. We report the correlates of S1-alpha power in these meditators' self-reports and the cognitive processes they identified to most effectively modulate S1-alpha band activity. Initial EEG data (n=6) suggests that hypothesis blind participants using this S1-alpha NIMT demonstrate increased alpha-band power. In the next phase of this experiment, we will compare the influence of these techniques on EEG-recorded S1-alpha power and coherence, as well as on behavior that has been previously associated with S1-alpha activity—i.e. threshold levels for detection of tactile stimuli (Kerr 2011). These preliminary results suggest that the NIMT methodology may

offer neurofeedback-independent modulation of targeted neurophysiological signals with important implications for clinical treatment.

Disclosures: **J.F. Santoyo:** None. **B. Cullen:** None. **C. Kerr:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH R01 NS055829

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Gustavus and Louise Pfeiffer Research Foundation

Title: Broadband gamma activity in the formation of a conscious visual experience in humans

Authors: ***W. R. XIAO**[1,2], S. I. KRONEMER[2], L. M. GOBER[1], R. E. SMITH[1], S. M. A. WAFA[1], A. RAJA[1], E. C. MORSE[1], R. E. WATSKY[1], C. L. HORIEN[1], E. SABERSKI[1], O. MORGAN[1], G. J. TOULOUMES[1], W. C. CHEN[1], D. D. SPENCER[3], J. L. GERRARD[3], H. BLUMENFELD[1,2,3];
[1]Neurol., [2]Neurosci., [3]Neurosurg., Yale Univ., New Haven, CT

Abstract: We used human electrocorticography to directly observe the temporal progression of neural activity during conscious visual events. We developed a threshold perception task paradigm using a face image held at 50% perceptual levels. We recruited 9 patients from the Yale Epilepsy Surgery Program undergoing observation for intractable epilepsy to perform our task by reporting perception of the image verified by reporting its location. Each patient was implanted with 100-300 subdural and depth electrodes sampled at 1024 Hz. We calculated the z-score of 40-115 Hz gamma power changes in each electrode as a measurement of local neuronal population spiking. Gamma power increases were observed in the primary visual cortex for all trials immediately following stimulus presentation. When perceived, however, by 300 ms post-stimulus the primary visual cortex showed gamma decreases along with strong visual association area increases. Those increases were in turn followed by sustained increases in fronto-parietal association areas as well as deactivations in some traditionally identified default mode network areas such as the precuneus, ventral medial frontal, lateral parietal, and lateral temporal cortices.

By 900 ms post-stimulus, the primary visual and orbitofrontal cortices showed reactivation. When viewed grossly, the "wave" of activity responsible for conscious visual processing moves forward from the visual cortex along the dorsal and ventral streams. Early primary visual cortex activation followed by deactivation and culminating in reactivation is a novel observation. In contrast during not perceived trials, visual cortex increases were sustained with some more low-amplitude increases radiating into the dorsal and ventral visual stream. K-means clustering of time-series produced functional brain networks: 1) primary visual and higher-order association cortex, 2) early and 3) later intermediate association cortex and memory areas and 4) default mode network in perceived trials but not in not perceived trials. All clusters have high within-network integrity demonstrated by high silhouette values. The same clusters applied to not-perceived time-series show negative silhouette value means except for the visual cortex. We observed the formation of a conscious visual experience in the human brain from intracranial electrophysiology. In consciously perceived trials, an ordered pattern of activation and deactivation in functional networks emerged whereas strong activity was only observed in the primary visual cortex for not perceived trials, suggesting that concerted network activity, however transient, is a mechanism of conscious perception.

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Poster

646. Human Cognition: Attention II

Location: Halls B-H

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Program#/Poster#: 646.13/LLL64

Topic: H.02. Human Cognition and Behavior

Title: Neural correlates of auditory attention in an exogenous orienting task

Authors: *M. SCARPETTA, M. PITTS, E. CANSECO-GONZALEZ;
Psychology, Reed Col., Portland, OR

Abstract: In an exogenous orienting task, attention is increased to the target stimulus if the cue validly predicts the target's location and the cue and target occur in quick succession. With a longer interval between the cue and target, the opposite effect occurs: attention is inhibited for validly cued targets. These attentional phenomena are known as facilitation, and inhibition of return (IOR), respectively. Both effects have been extensively explored in vision but less so in the auditory domain. The visual N2pc, an attention-related event-related potential (ERP)

component has been used to examine the neural correlates of IOR (McDonald et al., 2009; Yang et al., 2012), but recently, an auditory analog of the N2pc was discovered, known as the N2ac (Gamble & Luck, 2011). To our knowledge, no previous study has explored the neural basis of exogenous attentional facilitation and IOR in the auditory modality. The present study sought to fill this gap using the N2ac as a neural marker of auditory spatial attention. Brain activity was recorded from nineteen participants while they performed a Posner exogenous auditory orienting task. We compared the ERPs elicited by the target stimulus for short (200 ms) cue-to-target intervals (facilitation), and long (700 ms) cue-to-target intervals (IOR). We observed behavioral and electrophysiological evidence of attentional facilitation, and a behavioral trend of IOR, but no apparent electrophysiological evidence of IOR. This study demonstrates that the N2ac is enhanced by exogenous attention during the facilitation phase of the cue-to-target interval, but remains unaffected during the later IOR phase. These findings suggest some similarities as well as some differences between this newly discovered ERP component (N2ac) and its visual analog, the N2pc.

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Poster

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Topic: H.02. Human Cognition and Behavior

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Title: A decision-theoretic model of the temporal dynamics of visual priming

Authors: ***H. ZHANG**[1], Y. HUANG[2], H. LUO[1];
[1]Peking Univ., Beijing, China; [2]Shenzhen Inst. of Advanced Technology, Chinese Acad. of Sci., Shenzhen, China

Abstract: It is widely acknowledged that brain activities are intrinsically dynamic. Interestingly, recent studies also reveal rich temporal fluctuations in behavioral performances. For example, a visual priming study (Huang et al., 2015) sampled priming behavioral performances (i.e., reaction times, RT) at varied SOA with an unusually high sampling rate (50 Hz) and found that the resulting RT function could be decomposed into two components: a slow declining trend that is matched with classical priming pattern and a new theta-band (~4 Hz) periodical pattern.

Here we investigated whether the newly observed theta-band rhythm in priming behavior is hard-wired, or could be influenced by the rewarding structure of the environment. In particular, we manipulated temporal uncertainty of the target appearance after prime onset. In previous experimental studies, SOA and temporal uncertainty usually co-varied with each other. For example, at a later SOA, it is more certain that the target would come at the next moment, and vice versa. In contrast, we designed an experiment in which SOA and temporal uncertainty could be disentangled. Thus we could compare human subjects' RTs across conditions where SOAs were the same but temporal uncertainties were different, or the reverse.
We found that the manipulation of temporal uncertainty exerted prominent effects on the slow-trend component but more subtle effects on the oscillation component. Some of the effects are counterintuitive and most effects cannot be predicted by any existing theories of priming. We developed a decision-theoretic model to account for the effects of uncertainty on RT. The model also provides a new perspective for widely reported but unexplained phenomena in other fields such as attention.

Disclosures: H. Zhang: None. **Y. Huang:** None. **H. Luo:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH R01 NS055829

Title: Machine learning to predict pupillary dynamics in conscious visual perception

Authors: *S. M. A. WAFA[1], B. KIELY[1], W. R. XIAO[1,2], Y. CHEN[1], L. M. GOBER[1], J. PRINCE[1], S. I. KRONEMER[1,2], E. SABERSKI[1], O. MORGAN[1], Z. DING[1], M. J. MCGINLEY[2], D. A. MCCORMICK[2], H. BLUMENFELD[1,2,3];
[1]Dept. of Neurol., [2]Dept. of Neurosci., [3]Dept. of Neurosurg., Yale Univ., New Haven, CT

Abstract: The neural mechanisms of consciousness are the focus of many philosophic and empiric studies. Conscious perception requires attending to transient stimuli and encoding them in short-term memory for potential subsequent report. Recent data indicates that conscious perception might be associated with cortical and subcortical attention networks and memory encoding systems. A challenge for studying consciousness is the necessity of overt responses in behavioral paradigms. To address this limitation, we explored the use of pupillometry to covertly measure conscious visual perception. Pupillary responses have been correlated with several

cognitive processes in humans and more recently have been associated with auditory signal detection in rodents. We measured pupil diameter continuously using a pupillometry system during a visual threshold perception task in which face stimuli were calibrated to each participant's 50% perception threshold, confirmed by response accuracy. Changes in pupil dilation were measured and compared between perceived and not-perceived trials. On average, perceived trials were associated with an increase in pupil diameter approximately 1-2 seconds post-stimulus and this response was not seen in the average data for the not-perceived trials. Machine learning methods suggest that pupillary responses in individual trials can be predicted with an accuracy of 76.30% ± 13.46 for perceived and 78.25% ± 13.05 for not-perceived trials. This study may offer a new paradigm to gauge awareness without overt report. In addition, the results may enhance scientific knowledge on conscious information processing and inform the diagnosis of neurological disorders.

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Poster

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Program#/Poster#: 646.16/LLL67

Topic: H.02. Human Cognition and Behavior

Title: Attending to auditory targets enhances visual perceptual processing in ventral visual cortex

Authors: *K. M. SWALLOW, H. B. TURKER, R. MOYAL, B. G. LI;
Psychology, Cornell Univ., Ithaca, NY

Abstract: Although attention is limited, there is at least one circumstance under which attentional capacity appears to increase in response to increased task demands. In the attentional boost effect, encountering an auditory tone (e.g., a low tone) that requires a behavioral response (e.g., a button press) is associated with better memory for concurrently presented visual images than is encountering a tone that requires no response (e.g., a high-tone distractor). In this study we asked whether increasing attention to a target tone facilitates perceptual processing of concurrent visual information by improving the signal-to-noise ratio (SNR) within visual cortex. We measured BOLD activity while participants monitored individually presented auditory tones for pre-specified targets (auditory tone detection task) and simultaneously memorized briefly presented, masked visual images (500 ms duration). Participants pressed a button as soon as they

heard a target tone and did nothing when they heard a distractor tone (target tone pitch was counterbalanced across runs) or when they heard no tone. Univariate analyses showed that auditory target detection increased BOLD activity in visual cortex relative to distractor tones and no-tones. Of primary interest, however, was whether it also improved the SNR in visual cortical areas. In an MVPA analysis, we therefore asked whether auditory target detection improved the ability to classify which type of image was presented on a given trial based on patterns of activation within primary visual cortex, the fusiform face area (FFA), and the parahippocampal place area (PPA). If auditory target detection improves the SNR in visual cortex, classification should be better for images presented with auditory targets than images presented on their own or with a distractor in the auditory tone detection task. Consistent with our predictions, the types of images that were presented on a given trial were more accurately classified if the image was presented with an auditory target tone, rather than an auditory distractor tone or on its own. This was true for the FFA and PPA as well as for early visual cortex. In contrast to the interference effects that are typically observed during divided attention tasks, our data demonstrate that increasing attention to a behaviorally relevant stimulus in the auditory modality can improve information processing in visual areas of the brain. H.T. & R.M. contributed equally to this project.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: Natural Sciences and Engineering Research Council of Canada and the Canada Foundation for Innovation

EU Grant PIOF-GA-2012-329920

Title: Sounds reflexively decrease the occipital alpha rhythm: disentangling voluntary and involuntary shifts of cross-modal attention

Authors: *V. S. STOERMER[1], A. T. WALLACE[2], S. A. HILLYARD[3], J. J. MCDONALD[2]; [1]Psychology, Harvard Univ., Cambridge, MA; [2]Simon Fraser Univ., Vancouver, BC, Canada; [3]Univ. of California San Diego, San Diego, CA

Abstract: A salient, peripheral sound can influence visual processing of a subsequent target at the same location. Recently, we discovered that a sound elicits activity over occipital scalp sites even in the absence of visual stimulation, suggesting that a sound activates visual cortex automatically. In particular, we found that a peripheral sound triggers a slow positive deflection over contralateral occipital cortex (the ACOP) that is accompanied by a decrease of the occipital alpha rhythm (8-14Hz) - a brain oscillation that has been linked to visual-spatial attention. Here we explore to what extend these sound-induced changes in occipital alpha activity depend on the voluntary deployment of attention. A salient noise burst was presented on the left or right side and was followed by a masked visual target 1200 ms later. In different conditions, the target always appeared at the same location as the sound (predictive cue) or at a mirror-symmetric location on the opposite side of fixation (counter-predictive cue). Participants were asked to attend to the anticipated target location and to discriminate the identity of the target as quickly as possible. Consistent with the hypothesis that sounds reflexively enhance activity in contralateral visual cortex, alpha-band activity measured over the contralateral occipital scalp dropped ~200 to 500 ms after cue onset in both conditions. While this initial alpha reduction was greater contralateral than ipsilateral for both predictive and counter-predictive sounds, these conditions showed an opposite pattern of contralateral and ipsilateral activity in the later time interval, consistent with the voluntary allocation of attention to the anticipated target location. Overall, these data show that a salient, lateralized sound triggers an initial decrease of the contralateral occipital alpha rhythm regardless of its predictive value, supporting the hypothesis that a salient sound captures attention involuntarily and enhances visual activity in a spatially specific manner.

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Poster

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Program#/Poster#: 646.18/LLL69

Topic: H.02. Human Cognition and Behavior

Title: Predictions, not attention, modulate the first feedforward sweep of cortical information processing

Authors: *H. A. SLAGTER[1], J. ALILOVIC[2], B. TIMMERMANS[2], L. C. RETEIG[2]; [1]Dept. of Psychology, [2]Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: Both attention (stimulus relevance) and expectation (stimulus probability) have been shown to alter information processing in primary visual cortex, suggesting that top-down

influences can modulate the very first stage of cortical information processing. However, prior work either confounded attention and expectation, rendering their specific effects unclear, or used fMRI, which has low temporal resolution, leaving it unclear if these effects reflect a modulation of the first feedforward sweep of visual information processing or later, feedback-related activity. The current study orthogonally manipulated stimulus relevance and likelihood while exploiting the high temporal resolution of EEG to investigate if attention and/or expectation can modulate initial afferent activity in V1, as indexed by the early C1 component. Because the C1 is highly variable across individuals, for each participant we first determined two spatial locations at which the C1 could be reliably measured. Next, subjects performed an attentional cuing task in which they were cued on a trial-by-trial basis to direct their attention towards one of these locations and press a button whenever a target stimulus was presented at that location. The probability of a stimulus appearing at a given location was manipulated block-by-block, such that in different blocks the likelihood that a stimulus would appear was high (75%), neutral (50%) or low (25%). ERP analyses revealed that only stimulus probability, not stimulus relevance, had an effect on the amplitude of the C1. Specifically, the C1was largest for predicted stimuli and smallest for unpredicted stimuli. These results may indicate an effect of expectation, not attention, on the first feed-forward sweep of information processing.

Disclosures: **H.A. Slagter:** None. **J. Alilovic:** None. **B. Timmermans:** None. **L.C. Reteig:** None.

Poster

646. Human Cognition: Attention II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 646.19/LLL70

Topic: H.02. Human Cognition and Behavior

Support: Mind and Life Francisco J Varela Award

Title: An open-source system for EEG-neurofeedback and transcranial current stimulation control: Applications for clinical treatments

Authors: ***B. CULLEN**[1], J. SANTOYO[2], C. KERR[3], C. MCFARLANE-BLAKE[4];
[2]Neurosci., [3]Family Med., [1]Brown Univ., Providence, RI; [4]Boston, MA, Boston, MA

Abstract: We present an open-source EEG hardware and software system for neurofeedback and transcranial current stimulation (tCS) control and discuss its applications for clinical research. Neurofeedback and tCS are non-invasive neuromodulation techniques with demonstrable utility in therapeutic contexts. These techniques allow for the volitional modulation of specifically

targeted neurophysiological signals. Our open-source system offers a cost-efficient approach to EEG and neurofeedback research and allows for customizability to specific research programs. Additionally, this system provides an interface for closed-loop tCS paradigms, wherein specific changes in selected neurophysiological signals can be set to trigger tCS stimulation. This allows for rigorous experimental control and versatility in real-time EEG-based neurofeedback paradigms.This combined neurofeedback and tCS package offers a possibility of supplementing existing neuromodulation techniques and has utility in neuroscience research that seeks to use objective tools of measurement to explore the neurophysiological correlates of first-person experience, e.g. pain sensations. We demonstrate examples for these applications with data that represents successful modulation of somatosensory alpha-band power and by elucidating correlations between changes in EEG recorded alpha-band activity and expert meditators' self-report while viewing a neurofeedback display of somatosensory alpha-band activity.

Disclosures: **B. Cullen:** None. **J. Santoyo:** None. **C. Kerr:** None. **C. McFarlane-Blake:** None.

Poster

646. Human Cognition: Attention II

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Program#/Poster#: 646.20/MMM1

Topic: H.02. Human Cognition and Behavior

Support: MRC Grant MR/J009024/1

Title: The neural basis of reward-driven attentional capture

Authors: *L. TANKELEVITCH[1,2], E. SPAAK[1,2], M. F. S. RUSHWORTH[1], M. G. STOKES[1,2];
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Abstract: Selective attention is the prioritization of information by relevance, which is often defined by task goals (e.g., searching for food), or by the intrinsic salience of stimuli (e.g., a bright flash). Importantly, attention can also be driven by reinforcement learning, in which stimuli with a history of reward can capture attention in contexts in which they are not task-relevant, intrinsically salient, or rewarded. Here we show that human subjects' response times and discrimination performance are affected by reward-driven attentional capture, and probe the neural dynamics of these effects.
First, subjects completed a learning task in which they aimed to maximize their monetary rewards by choosing between visual tokens, each associated with a certain reward outcome.

They then completed a visual discrimination task, in which they had to report the orientation of a lateralized target grating, while ignoring a distracter grating. Importantly, subjects also encountered the previously rewarded - but now irrelevant - tokens appearing around the gratings. A low or high value token could appear around the target (congruent trials) or around the distracter grating (incongruent trials). To investigate the timecourse of reward-driven attention, the tokens appeared at 1000, 500, or 0 ms delays relative to the gratings.
Subjects' response times were slower in incongruent and faster in congruent trials, relative to a neutral condition in which only zero-value tokens were encountered. Moreover, subjects were also worse at discriminating the target in incongruent trials. Critically, these effects were modulated by reinforcement learning, occurring only for the high value tokens, and appeared in all delay conditions.
In parallel, we used magnetoencephalograpy (MEG) to record brain activity as participants completed both tasks. Focusing on the discrimination task, preliminary results show that alpha-band (8-12 Hz) lateralization, an index of spatial attention, is modulated by the presence of task-irrelevant but previously high-value tokens. Moreover, reward history information is decodable from the brain as early as ~250 ms after stimulus onset, and dissipates ~500 ms later. These results indicate that reward-driven attentional capture can be indexed by multiple neural measures of attentional allocation, and further our understanding of the interaction between reinforcement learning and selective attention.

Disclosures: L. Tankelevitch: None. **E. Spaak:** None. **M.F.S. Rushworth:** None. **M.G. Stokes:** None.

Poster

646. Human Cognition: Attention II

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Topic: H.02. Human Cognition and Behavior

Support: W81XWH-11-2-0044

Title: The role of confidence in self-reported mind wandering and task engagement: an fMRI investigation

Authors: E. DENKOVA, M. KRIMSKY, A. B. MORRISON, *A. P. JHA;
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Abstract: Mind wandering (MW), defined as off-task thinking that is self-generated and unrelated to the task at hand, has seen a recent escalation as a topic of interest in the cognitive

neuroscience literature. Recent neuroimaging studies investigate the neural underpinnings of MW, which has typically been assessed through subjective reports collected in response to probe questions embedded in a cognitive task. Yet, very little is known about the factors that may modulate the neural correlates underlying self-reports of MW. Here, we investigated the neural correlates of self-reported task engagement *vs.* MW during an attention task by considering participants' confidence in these self-reports. We collected functional Magnetic Resonance Imaging (fMRI) data from 38 participants while they completed the sustained attention to response task (SART) with embedded experience sampling probe-questions. The first question assessed participants' experience of task engagement *vs.* mind wandering using a dichotomous judgment of 'on task' *vs.* 'off task'. The second question assessed participants' level of confidence in their self-reports of 'on task' and 'off task' using a 4-point Likert scale. In the fMRI analysis, the 6s periods preceding the participants' response to the first probe question were modeled as a function of 'on task' *vs.* 'off task' reports, while confidence ratings were considered as a parametric modulator on a trial-by-trail bases. The fMRI results revealed that level of confidence in 'on-task' *vs.* 'off-task' reports differentially modulated dorsal *vs.* ventral lateral regions of the prefrontal cortex (PFC), respectively. Particularly, higher confidence in being 'on task' was associated with greater dorsolateral PFC, while higher confidence in being 'off task' was associated with ventrolateral PFC. These findings pinpoint the importance of considering the level of confidence as a form of meta-cognitive assessment in the investigation of the neural mechanisms underlying self-reports of task engagement and MW. The present study sheds light on the engagement of brain regions associated with executive control as a function of confidence in subjective reports.

Disclosures: **E. Denkova:** None. **M. Krimsky:** None. **A.B. Morrison:** None. **A.P. Jha:** None.

Poster

646. Human Cognition: Attention II

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Topic: H.02. Human Cognition and Behavior

Support: NIH/NCCIH grant R01 AT007922-01

UMass Medical School institutional funds

Title: Exploring markers for neurofeedback-based augmentation of effortless awareness meditation.

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[1]Preventive Med., Univ. of Massachusetts, Worcester, MA; [2]Univ. Med. Ctr., Utrecht, Netherlands; [3]MIT, Cambridge, MA; [4]VUmc, Amsterdam, Netherlands

Abstract: Introduction: Meditation has shown beneficial effects for multiple psychiatric disorders. However, learning to meditate is not straightforward as there are no easily discernible outward signs of performance and thus no direct feedback is possible. Real-time neurofeedback based on signals that are associated with effective meditation practice may provide a solution to this issue. We showed in a previous study that EEG source-estimated neurofeedback corresponds with the subjective experience of effortless awareness (which is a major component of meditation). In the present study, we aim to identify additional potential neurofeedback targets by comparing EEG activity between novice and experienced meditators during effortless awareness practice. These can then be tested in further neurophenomenology experiments for their temporal association with the subjective experience of effortless awareness and potential efficacy in increasing specificity of the EEG source-estimated neurofeedback signal.
Methods: Sixteen experienced meditators and sixteen age and gender matched novice meditators participated in the study. Experienced meditators performed a self-selected meditation practice that supported effortless awareness and novice meditators were briefly trained to perform a basic meditation practice. Eyes-open EEG recordings were obtained while participants meditated. Power in the theta (4-8 Hz), alpha (8-13 Hz) and beta (13-20 Hz) frequency bands was calculated for 19 EEG sensors covering the whole head. Mean functional connectivity per electrode was assessed using the phase-lag index (PLI). Differences between groups were assessed using repeated-measures analysis of variance (ANOVA).
Results: For alpha power, a significant interaction ($p=0.10$) was observed as well as a significant between group difference ($p=0.027$). Post-hoc testing showed increased power in the F3, P4, O2 and Pz electrodes in the experienced group compared to the novice group. Visual inspection revealed that median alpha power was higher in the experienced group for all 19 electrodes (range 29 – 204%, median 80%). For functional connectivity in the alpha band, a significant group difference ($p=0.009$) was observed. Post-hoc testing showed increased functional connectivity in the experienced meditator group compared to the novice group in 15 electrodes. Visual inspection revealed that median functional connectivity was higher in the experienced group for all 19 electrodes (range 9-24%, median 17%).
Discussion: Global alpha power and functional connectivity might be explored further as potential neurofeedback targets to augment effortless awareness.

Disclosures: R. Van Lutterveld: None. **E. van Dellen:** None. **S. Houlihan:** None. **P. Pal:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GOBLUE INTERNATIONAL, LLC. **K. Stam:** None. **J. Brewer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GOBLUE INTERNATIONAL, LLC.

Poster

646. Human Cognition: Attention II

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Topic: H.02. Human Cognition and Behavior

Support: NSERC Postdoctoral Fellowship

U.S. Army Research Office W911NF-09-0001

Title: Reconstructing location-selective population codes during the attentional blink

Authors: *M. MACLEAN[1,2], T. BULLOCK[1,2], B. GIESBRECHT[1,2];
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Abstract: Following the presentation of a target (T1) embedded in a rapid serial visual presentation, the ability to detect or identify a subsequent target (T2) is impaired for ~ 500 ms, an effect referred to as the attentional blink (AB; [Raymond, Shapiro, & Arnell, 1992]). Some evidence suggests that the locus of this effect is post-perceptual [Vogel, Luck, & Shapiro, 1998], such as a temporary loss of control over attention [Di Lollo, Kawahara, Ghorashi, & Enns, 2005]. In the current experiment we used an inverted encoding model to estimate location-selective channel tuning functions (CTFs) from scalp recorded EEG in the alpha-band for location information [Garcia, Srinivasan, & Serences, 2013] as a model for selective attention. If the AB affects early selectivity then the profile of the CTF should change during the AB period relative to the pre-T1 period. Participants (n = 4) observed a flickering Gabor presented in the periphery for 3 seconds. The Gabor briefly (100 ms) rotated either clockwise or anti-clockwise (T1), followed by a Gabor of a different orientation (T2) either 200 (lag 2) or 900 ms (lag 9) later. Participants reported the direction of the T1 rotation (CW/ACW), and then reported the orientation of T2 on a continuous scale. We modeled the degree of error in the T2 response [Bays & Husain, 2008; Zhang & Luck, 2009] and found that the precision of T2 report was reduced at lag 2 (k = 24°) as compared to lag 9 (k = 20°; p = .17), indicating the presence of an AB effect. We calculated average slope and amplitude of the location-selective CTF for three time points: pre-T1 (-800-0 ms), immediately post-T1 (0-200 ms), when lag-1 sparing is observed [Di Lollo et al., 2005], and during the AB (200-400 ms). We did not observe a decrement in either the slope (*pre-T1* = .02 μV^2/10°, *SE* = .002; *lag 1* = .02, *SE* = .009; *AB* = .03, *SE* = .009) or the amplitude (*pre-T1* = .14 μV^2, *SE* = .02; *lag 1* = .19, *SE* = .04; *AB* = .17, *SE* = .06) of the location-selective CTF profile during the AB. Furthermore, amplitude of the CTF was greater during the lag-1 period, relative to the pre-T1 period (p = .10). This increase fits with the theoretical account of an initial “boost” of attention following T1, also thought to cause lag-1

sparing [Olivers & Meeter, 2008]. Our results indicate that the selective representation of location information was not disrupted during the AB period.

Disclosures: **M. Maclean:** None. **T. Bullock:** None. **B. Giesbrecht:** None.

Poster

646. Human Cognition: Attention II

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Topic: H.02. Human Cognition and Behavior

Title: Neurophysiological correlates of deliberate and spontaneous mind-wandering

Authors: *A. MARTEL[1], M. ARVANEH[4], P. DOCKREE[2], I. ROBERTSON[3];
[2]Sch. of Psychology, [3]Trinity Institue of Neurosci., [1]Trinity Col. Dublin, Dublin, Ireland; [4]Dept. of Automatic Control and Systems Engin., Univ. of Sheffield, Sheffield, United Kingdom

Abstract: *Motivation:* Recent years have witnessed a substantial increase in the number of neuroscientific studies investigating mindwandering, i.e. selfgenerated, taskindependent thoughts. Latest findings suggest that mindwandering is a heterogenous class of mental states and many authors now argue in favor of the differentiation of mindwandering (MW) with and without awareness (Smallwood and AndrewsHanna 2013; Seli et al. 2015; Fox et al. 2015) . The former being described as an intentional, controlled or deliberate MW (dMW) and the latter as unintentional or spontaneous MW (sMW). *Aim:* The aim of the present study was to identify neurophysiological differences between sMW and dMW. *Material and Methods:* 26 subjects (12 female; 23.4 ± 3.2 years) performed two tasks, a breathcounting (BC) task and a fixed version of the SART (Robertson et al. 1997) while electroencephalography (EEG) data was recorded. MW was assessed in both tasks with 5scale selfinitiated or randomly interrupting thought probes with the following options: Attention was (1) ontask (no mindwandering), (2) on thoughts pertaining to the task, (3) distracted by internal sensations or external distractions, (4) on reminiscing or planning thoughts (dMW), (5) daydreaming (sMW). *Results :* Behavioral results revealed significant differences in response time coefficients of variability (RTCV) for grouped trials leading up to reports of focused attention, dMW and sMW during the SART. Analysis of the 64-channel EEG recorded data revealed that average α activity prior to thought probes differentiates between the ontask and sMW conditions as well as between dMW and sMW conditions. Time-frequency analysis revealed increased activity in the α band and decreased activity in the lower- ;;, ;; and lower- ;; bands between dMW and sMW for both tasks. Eventrelated potentials (ERPs) for the SART trials preceding MW reports exhibited significant differences across all conditions. P3a components gradually decreased in amplitude for SART trials leading up to

dMW and sMW reports when compared to ontask reports with the most pronounced decrease for the sMW condition. These results demonstrate that both dMW and SMW have distinct behavioral and neurocortical signatures.

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Poster

646. Human Cognition: Attention II

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Support: NIH EB00856

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Title: Interactions between prefrontal cortex and temporal lobe during selective attention in a cocktail party task

Authors: *A. DE PESTERS[1,2], P. BRUNNER[1,3], G. SCHALK[1,2,3];
[1]Natl. Ctr. for Adaptive Neurotechnologies, Albany, NY; [2]State Univ. of New York at Albany, Albany, NY; [3]Dept. of Neurol., Albany Med. Col., Albany, NY

Abstract: The cocktail party effect describes the ability to focus on one of several simultaneous auditory streams. Recent studies have established that cortical activity in specific frequency bands and cortical locations selectively tracks the attended auditory stream (Mesgarani and Chang, 2012; Golumbic et al., 2013), but the mechanisms that support this function have been unclear.
It is well established that the prefrontal cortex (PFC) plays a crucial role in the top-down modulation of attention, in particular during complex cognitive tasks (Zanto et al., 2012; Voytek et al, 2015). In addition, previous studies have shown that variations in oscillatory activity in the alpha band (8-12 Hz) may implement top-down modulation of cortical activity by increasing excitability in task-related areas and inhibiting it in task-unrelated ones (Klimesch et al., 2007; de

Pesters et al., 2016). Based on this evidence, we hypothesized that the PFC may be involved in selective auditory attention. Specifically, we hypothesized that the PFC receives task-relevant information from early auditory cortex and perisylvian areas, and may use this information to exert modulatory influences of cortical excitability in those same areas.
To investigate the interplay between bottom-up sensory input and top-down modulation of auditory attention, we recorded electrocorticographic (ECoG) activity from subdural electrode grids in ten human subjects during a cocktail party task. The subjects were asked to focus on one of two simultaneously presented speakers. Our results demonstrated the existence of two distinct populations of cortical locations within perisylvian areas. The first subset of locations responded mostly to the attended speaker, while the second subset responded to both attended and unattended speakers, thereby confirming the findings from previous studies (Golumbic et al., 2013; Dijkstra et al., 2015). More importantly, we found that locations from both subsets of auditory locations modulated PFC population-level cortical activity in the broadband gamma (> 70 Hz) range. In turn, cortical excitability (as measured by the power in the 8-12 Hz band) in those same subsets was modulated by population-level cortical activity in the PFC. These interactions were specific to the attended speaker.
In sum, our results suggest that the PFC receives information about the attended auditory stream from auditory cortical areas, and that the PFC in turn modulates cortical excitability in auditory cortical areas. We expect that these results help to clarify our understanding of the relationship between PFC and auditory areas during selective auditory attention in a cocktail party.

Disclosures: **A. De Pesters:** None. **P. Brunner:** None. **G. Schalk:** None.

Poster

646. Human Cognition: Attention II

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Topic: H.02. Human Cognition and Behavior

Title: Alpha desynchronization during a visual-spatial attention task in concussed athletes.

Authors: *S. GUAY[1], L. DE BEAUMONT[1], P. JOLICOEUR[2];
[1]Dept. de psychologie, Univ. Du Québec À Trois-Rivières, Trois-Rivières, QC, Canada; [2]Univ. de Montréal, Montreal, QC, Canada

Abstract: ***Objective*:** To report Alpha amplitude/power baseline and investigate event-related spectral perturbations (ERSPs) in alpha-band activity in multiply concussed athletes relative to unconcussed athletes.
Background: Event-related potentials (ERPs) have been useful to detect persistent subclinical

alterations of cognitive processes in concussed athletes. Studies interested in ERP markers of attention revealed significant N1, P2 and P3 amplitude alterations in asymptomatic concussed athletes. Pertinently, concussion effects on these ERP markers of attention and working memory were found to be exacerbated with recurrent concussions.
***Design/method*:** A total of 45 university football players were assigned to three experimental groups based on prior concussion history: Athletes with a single-concussion history (n = 15); Athletes with two or more concussions (n = 15, M = 2.8, SD = 1.32); and a control group of non-concussed athletes (n = 15). Time since last concussion was more than nine months and the average post-concussion time was two years. We studied the long-term and cumulative effects of concussion on alpha-frequency Event-related desynchronization (ERD) when performing a visuospatial attention task.
***Results**:* This study found that the baseline Alpha amplitude/power was significantly lower in the multi-concussion group compared to the control group, $F(1,29) = 4.54$, $p = .04$. After correction for baseline alpha activity, Alpha ERD was still significantly reduced among the multi-concussion group ($F(1,29) = 8.03$; $p = .009$) and the latter was negatively associated with baseline Alpha ($r(24) = -.786$, $p = .002$).
Conclusion: This finding suggests that recurrent concussions significantly alter Alpha rhythm ERD elicited by a visual-spatial attention task. Baseline Alpha activity was found to strongly predict subsequent Alpha ERD amplitude in athletes with recurrent concussions. Baseline alpha activity and Alpha ERD disruptions could underlie known attention-related ERP alterations in recurrent concussion athletes.

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Poster

646. Human Cognition: Attention II

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Topic: H.02. Human Cognition and Behavior

Support: University of Texas Undergraduate Research Fellowship (to TBN)

Title: Development of a closed-loop feedback system using eye-tracking technology to train sustained attention

Authors: *K. E. SELOFF[1], T. B. NGUYEN[2], J. L. MOSES[3], C. G. BEEVERS[3], D. M. SCHNYER[3];
[1]Inst. for Neurosci., [2]Neurosci., [3]Psychology, Univ. of Texas At Austin, Austin, TX

Abstract: Sustained attention is critical in everyday functioning and has been shown to be limited, with healthy individuals showing a decrease in sustained attention after as little as 5 minutes. Previous work using closed-loop real-time fMRI neurofeedback to improve sustained attention demonstrated that providing feedback on attentional focus by altering the display saliency was effective in improving sustained attention on a real-time Attention Training version of the Sustained Attention to Response Task (rtAT-SART). This paradigm rewarded participants for sustained attention by making the task easier and alerted participants to lapses in attention (ideally before those lapses affected task performance) by making the task more difficult. We are conducting a preliminary investigation of a closed-loop eye-tracked Attention Training version of the SART (etAT-SART) that modifies the rtAT-SART to use gaze data obtained from an eye-tracker instead of neural data obtained from rtfMRI as the basis for capturing attentional focus and adjusting feedback. Our preliminary study included 32 healthy undergraduate students, 12 of whom received real-time feedback based on whether or not they were attending to the target location during the etAT-SART and 20 of whom served as no-feedback controls. Preliminary results have shown that gaze accuracy (percentage of time on the instructed target image, as opposed to the distractor image) improves in response to feedback (mean improvement of 11.8% from the pre-training test), but does not change in no-feedback controls (mean improvement of 0.9% from the pre-training test) ($t(13.51) = 4.34$, $p < 0.001$, Cohen's $d = 1.7$). This suggests that visual search patterns are altered in the presence of gaze-based feedback. Additionally, task performance on the SART shows a trend for improvement from the pre-training test to the post-training test in the feedback group (normalized A' change score of 0.019), but not in the no-feedback control group (normalized A' change score of 0.001) ($t(17.59) = 1.44$, $p = 0.169$). This suggests that gaze-based feedback can improve sustained attention similarly to its neurofeedback predecessor. We plan to analyze the fixation and scan path data from the eye-tracking system in a post-hoc manner to determine the optimal method of classifying the attended image in the etAT-SART. We will then investigate the use of this classification method as the basis for providing feedback in our closed-loop system. Once the task has been optimized, we plan to examine whether it can be used with negative stimuli (e.g. sad faces) as distractors to decrease negative attention bias in individuals with elevated depressive symptoms.

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Poster

646. Human Cognition: Attention II

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Program#/Poster#: 646.28/MMM9

Topic: H.02. Human Cognition and Behavior

Title: Cortical and subcortical involvement in switching between task positive network and default mode network

Authors: *R. LI[1,4], D. KLUGER[1], E. LEVINSOHN[1], Y. CHEN[1], W. XIAO[1], H. BLUMENFELD[1,2,3];
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Abstract: The human brain is organized into dynamic correlated and anti-correlated functional networks. Cognitively demanding tasks have consistently been shown to evoke activation of the brain's task positive network (TPN) and induce deactivation in the default mode network (DMN).However, the neural mechanisms underlying this switching between activation and deactivation of brain networks are not completely understood. Here, we used task functional magnetic resonance imaging (fMRI) to investigate the switch-like mechanisms in the brain that turns these networks on and off. We acquired task-fMRI data of 22 normal children (ages 11-16) completing a block-design continuous performance task (CPT). The average percent signal changes across all the blocks and subjects were calculated to examine brain changes during the switching process. In addition, we performed time course analyses for regions of interest (ROIs) extracted from the TPN and the DMN to describe the switching changes occurring with onset and offset of task. We observed significant activation of a network comprising the precuneus, anterior insula, supplementary motor area, medial thalamus, and midbrain during both onset and offset of task. Although the general view is that the TPN and the DMN are anti-correlated and have sustained activation or deactivation during task, our results suggested that some regions within the TPN and the DMN show increased activity both as a response to the onset of task and as a response to the offset of task. Moreover, we demonstrated a potential role for subcortical structures in mediating the switch between passive fixation and active task performance. Our findings provide new insights into the network switching mechanisms between brain networks involved in externally oriented attention and internally oriented mental processes.

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Poster

647. Behavioral Demonstrations of Executive Function

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Support: CONICYT National PhD Grant 21140175

Title: Effects of a brief mindfulness-meditation intervention on neural measures of response inhibition and performance monitoring in cigarette smokers

Authors: *C. I. ANDREU[1], D. COSMELLI[1,2], H. A. SLAGTER[3,4], I. H. A. FRANKEN[5]; [1]Escuela de Psicología, Pontificia Univ. Católica De Chile, Santiago, Chile; [2]Ctr. Interdisciplinario de Neurociencias, Pontificia Univ. Católica de Chile, Santiago, Chile; [3]Dept. of Psychology, [4]Amsterdam Brain and Cognition, Univ. of Amsterdam, Amsterdam, Netherlands; [5]Dept. of Psychology, Educ. & Child Studies, Erasmus Univ. Rotterdam, Rotterdam, Netherlands

Abstract: Research suggests that mindfulness-practices may aid smoking cessation. Yet, the neural mechanisms underlying the effects of mindfulness-practices on smoking are unclear. Response inhibition and performance monitoring are main deficits in addiction, associated with relapse. Notably, both functions are candidate targets for mindfulness-based practices. Using electroencephalography (EEG), the current study therefore investigated the effects of a brief mindfulness-practice on both functions in smokers. Participants underwent a cigarette cue-exposure, and were then randomly assigned to a group receiving mindfulness instructions or to a non-instruction control group. Then, they performed a smoking Go/Nogo and Flanker task, while their brain activity was recorded. No group differences in task performance were observed. However, EEG analyses revealed a decrease in Nogo-P3 amplitude in the mindfulness versus control group. As both groups performed equally well, the reduced P3 might indicate less-effortful response inhibition after the mindfulness-practice, and suggest that enhanced response inhibition underlies observed positive effects of mindfulness on smoking behavior.

Disclosures: C.I. Andreu: None. **D. Cosmelli:** None. **H.A. Slagter:** None. **I.H.A. Franken:** None.

Poster

647. Behavioral Demonstrations of Executive Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 647.02/MMM11

Topic: H.02. Human Cognition and Behavior

Support: Emory University Religion and Public Health Collaborative

Title: Cognition, Disinhibition and sexual risk behavior among African American adolescent females

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Abstract: Introduction: Adolescence is characterized by immature executive function and risk taking behavior. African American (AA) girls engage in higher sexual risk behavior (SRB) and are disproportionately affected by HIV and sexually transmitted infections (STIs), yet knowledge gaps persist regarding the role of neurocognitive determinants of AA girls' SRB.

Method(s): This cross-sectional study, guided by the Biopsychosocial Model of Risk Taking, examined the: 1) neuro-psychosocial profile of girls with high SRB vs low SRB and 2) association between depression, sensation seeking/disinhibition, cognitive function and SRB in a sample of 65 AA girls ages 15-23 years. Each participant and/or parent provided written informed consent and/or assent. Girls completed CANTAB computerized cognitive function tests, including the Information Sampling Task (IST) and Cambridge Gambling Task (CGT) and demographic, psychosocial and SRB surveys. Scores >1 on a SRB index (0-6) indicated high SRB. Bivariate correlations, ANOVA and regression statistics were used.

Results: Mean age was 17.8±1.9 years. Mean age at sexual debut was 15.5±2.6. Girls with high SRB (vs low SRB) were/had significantly: older (18.6 vs 16.9) and greater mean: depression (11.58 vs 7.26), disinhibition (4.29 vs 2.77) and lower mean: coping (165.14 vs 177.73) and accuracy on IST (poorer impulse control) (6.43 vs 7.38). Higher SRB scores were significantly ($p<.05$) associated with higher: age ($r= .41$), disinhibition ($r=.34$), depression ($r=.33$), boredom susceptibility ($r=.27$) scores and poorer/lower: coping ($r=-.26$), seeking spiritual support ($r=-.29$) and impulse control: IST sampling errors ($r=.30$), IST total correct ($r=-.30$), IST mean # boxes opened/trial ($r=-.32$). Some AA girls with high reported SRB (compared to low SRB) showed greater cognitive delay aversion during reward-related risk-taking (CGT). Girls with higher disinhibition were more likely to have higher SRB [$Exp(B)=3.08$, $p=.09$], while accounting for covariates (all non-significant). Greater disinhibition was also ($Beta= .47$, $p=.001$) associated with higher SRB index scores in a model accounting for 58.7% variance ($R2=.587$), beyond covariates (non-significant).

Discussion & Conclusions: Higher SRB was associated with disinhibition and poorer impulse control and poorer decision making, in our sample of AA girls. Those interested in helping AA girls to minimize their SRB should provide tailored HIV/STI prevention efforts based on important links between psychosocial factors, including disinhibition, and impulse control. Findings can be used to facilitate future imaging, longitudinal and intervention studies.

Disclosures: S.G. Dalmida: None.

Poster

647. Behavioral Demonstrations of Executive Function

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Topic: H.02. Human Cognition and Behavior

Support: NSERC Grant A2559

NIH Grant R21AG048431

Title: Brain signal variability in monolinguals and bilinguals

Authors: J. GRUNDY[1], *J. A. ANDERSON[2], E. BIALYSTOK[1];
[1]Psychology, York Univ., Toronto, ON, Canada; [2]Psychology, York Univ., Etobicoke, ON, Canada

Abstract: Bilinguals constantly use cognitive control to manage competition between languages that compete for selection (Green, 1998). Practice with this control might lead to domain-general enhancements in executive functioning (EF; Bialystok, Craik, & Luk, 2012). Support for this view has appeared on a range of EF tasks in both children (e.g. Barac, Bialystok, Castro, & Sanchez, 2014) and older adults (e.g. Bialystok, Craik, Klein, & Viswanathan, 2004) showing that bilinguals outperform their monolingual peers. Behavioral findings from young adults are more variable. In light of this, some have questioned whether second-language experience leads to domain-general cognitive changes (e.g. Paap & Greenberg, 2013). However, similar behavioral outcomes can emerge from quite different cognitive processes. This is especially true for young adults who often perform at ceiling on these tasks. The present study examined brain signal variability using multiscale entropy (MSE; Costa, Goldberger, & Peng, 2002) analysis. EEG was recorded while young adult monolinguals (N=20) and bilinguals (N=20) performed a task-switching paradigm. Brain signal complexity increases with development and is inversely related to reaction time (RT) variability (McIntosh, Kovacevic, & Itier, 2008). If lifelong bilingualism contributes to development of domain-general EF, then bilinguals should show greater brain signal variability while performing an EF task, even in the absence of mean RT differences. Results revealed that brain signal variability was greater for bilinguals than monolinguals in occipital, but not frontal electrode sites. Furthermore, signal variability predicted RT differently for bilinguals than monolinguals: Greater signal variability was associated with faster RTs in bilinguals but slower RTs in monolinguals. These findings suggest that bilinguals make use of brain complexity (i.e. signal variability) in a way that monolinguals do not.

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Poster

647. Behavioral Demonstrations of Executive Function

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Program#/Poster#: 647.04/MMM13

Topic: H.02. Human Cognition and Behavior

Title: Perceptual difficulty and its effect on response inhibition using the modified stop-signal paradigm

Authors: *M. V. SOLOVEVA[1], S. JAMADAR[1], G. POUDEL[1], M. HUGHES[2], N. GEORGIOU-KARISTIANIS[1];
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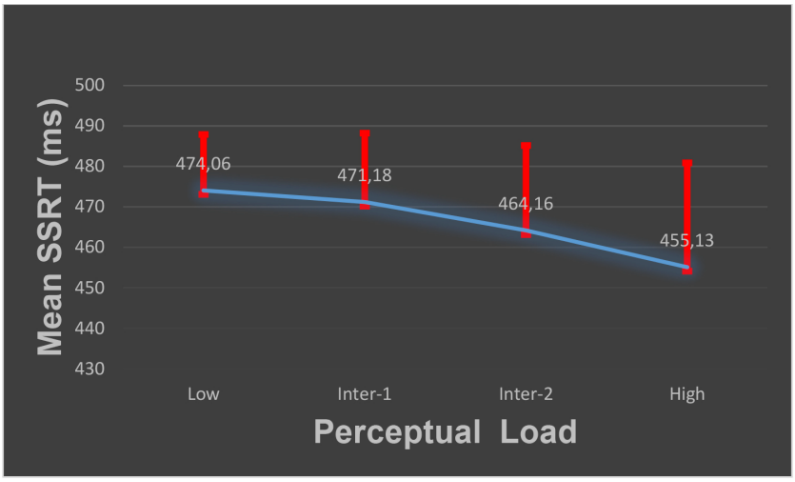
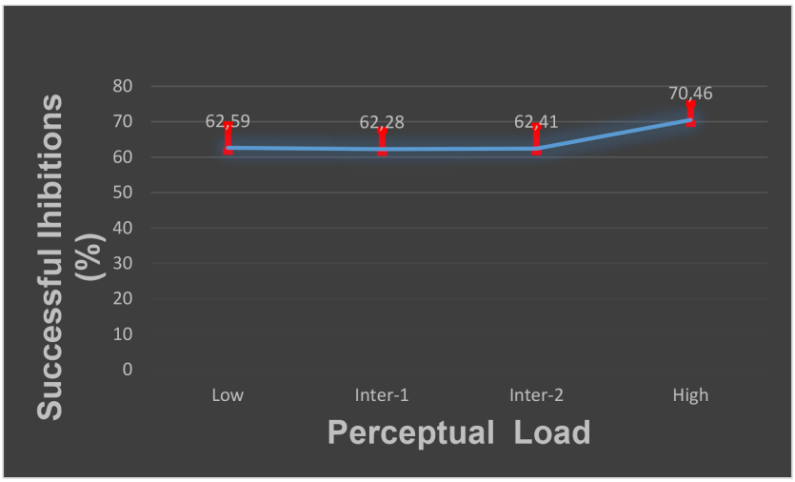
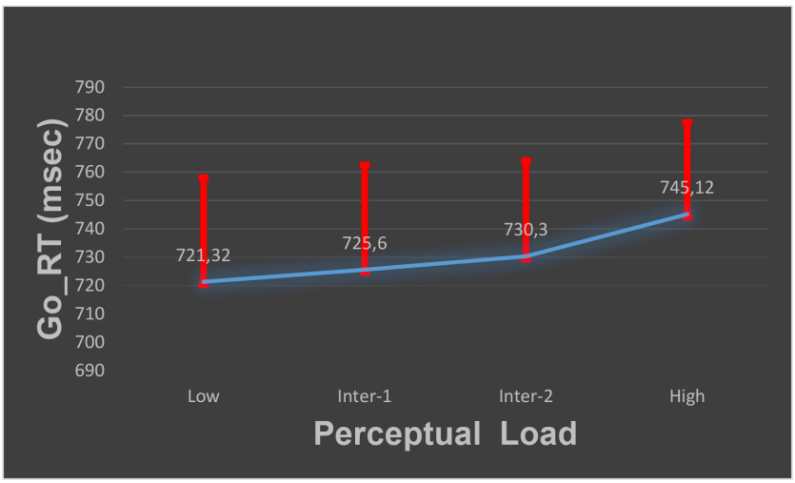
Abstract: The stop-signal paradigm has been widely used in behavioural research as a measure of response inhibition, a core component of cognitive control. It is known that stopping behaviour can be accounted for by assuming that a race between independent 'go' and 'stop' processes determine whether inhibition is successful or not. The interplay between the 'go' vs 'stop' processes and the quality of visual information and its impact on inhibition has also gained much interest in the literature. However, a few studies have examined how perceptual difficulty of the 'go' stimuli may indirectly affect the 'stop' process. In this experiment, we assessed how parametrically manipulating perceptual difficulty of the 'go' stimuli affects response inhibition, as measured by stop-signal reaction times (SSRTs) and probability of inhibiting (P_i). Eight healthy individuals (4 female and 4 male) (M = 30.00; SD = 6.00) underwent a modified 22-minute stop-signal task, where traditional 'go' stimuli (i.e., arrows) were replaced by images of 'Y' and 'V' letters with low, intermediate-1, intermediate-2, and high levels of perceptual clarity (levels of Gaussian blur). On 33% of trials, the stop-signal (50 ms audio tone) followed the 'go' stimuli after a brief interval (the stop-signal delay, SSD), which was individually adjusted for the participant to reach a minimum of 50% accuracy for the stop trials. Results showed that reaction times to the 'go' stimuli significantly increased with increased perceptual difficulty, $F(3, 18) = 6.07$, $p < .005$, = .50. No significant effect of perceptual difficulty on P_i and SSRTs was observed, however, a trend towards significance ($F(3, 18) = 2.51$, $p = .09$) was observed for Pi, indicating a trend towards higher probability of inhibiting as a function of perceptual difficulty. Our results indicate that 'go' and 'stop' processes are independent as assumed by the race model. These preliminary findings have implications for the relationship between the 'stop' and 'go' processes but require further validation if we are to disentangle how perceptual load may influence response inhibition.







Disclosures: **M.V. Soloveva:** None. **S. Jamadar:** None. **G. Poudel:** None. **M. Hughes:** None. **N. Georgiou-Karistianis:** None.

Poster

647. Behavioral Demonstrations of Executive Function

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Program#/Poster#: 647.05/MMM14

Topic: H.02. Human Cognition and Behavior

Title: Exploring human cognition research on a large scale through the Human Cognition Project

Authors: *K. KERLAN, N. F. NG, C. M. SIMONE, E. CORDELL, M. SCANLON;
Lumos Labs, Inc., San Francisco, CA

Abstract: Our growing understanding of human cognition has been impeded by conventional research methods involving many small-scale studies with local convenience samples at individual university laboratories utilizing incomparable task designs. These methods make cognition research slow, costly, and difficult to replicate. The Human Cognition Project (HCP) is a large-scale, online science project aimed to complement traditional laboratory-based experimental approaches used in the cognitive sciences. Inspired by Lumosity's web-based cognitive training platform that includes a suite of games, assessments, surveys, and tracking metrics, the HCP advances studies of cognitive training by supporting world-wide collaborations and offering researchers access to the largest database of human cognitive performance, with data from over 80 million individuals to date.
The HCP is guided by the hypothesis that bringing together a broad network of academic scientists and clinicians will accelerate investigations of cognitive functions such as processing speed, working memory, visual attention, and flexibility, thereby advancing the field of human cognition. Since the project formally began in 2011, HCP has resulted in twelve peer-reviewed publications. Ongoing studies include investigations of population trends in cognitive changes across the lifespan, neuroplasticity associated with targeted cognitive processes, and effectiveness of cognitive training as a neurotherapeutic tool for individuals affected by mild cognitive impairment, Multiple Sclerosis, stroke, ADHD, cancer, and TBI. Here we present an overview of the HCP science model as well as several primary findings from published studies that have resulted from the project.

Disclosures: **K. Kerlan:** A. Employment/Salary (full or part-time): Lumos Labs, Inc. **N.F. Ng:** A. Employment/Salary (full or part-time): Lumos Labs, Inc. **C.M. Simone:** A. Employment/Salary (full or part-time): Lumos Labs, Inc. **E. Cordell:** A. Employment/Salary (full or part-time): Lumos Labs, Inc. **M. Scanlon:** A. Employment/Salary (full or part-time): Lumos Labs, Inc..

Poster

647. Behavioral Demonstrations of Executive Function

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Topic: H.02. Human Cognition and Behavior

Support: NIH AA016624

SDSU

Title: Attentional modulation of response inhibition in a Go/No-Go task: spatio-temporal oscillatory dynamics in an anatomically constrained MEG model

Authors: J. P. HAPPER[1], L. C. WAGNER[1], L. E. BEATON[1], B. Q. ROSEN[2], *K. MARINKOVIC[1,3];
[1]Psychology, San Diego State Univ., San Diego, CA; [2]Neurosciences, [3]Radiology, UCSD, San Diego, CA

Abstract: Response inhibition is an integral aspect of cognitive control whose neural mechanisms have been studied extensively. However, the attentional aspects of response inhibition have not been fully elucidated. To investigate the effects of attentional capture on response inhibition, 22 healthy right-handed participants (10 female) performed a modified Go/No-Go task. Stimuli consisted of the letters 'X' and 'Y' where a Go stimulus was the alternation and a No-Go stimulus was the repetition of letters (20% of trials). Participants responded to Go stimuli using their right index finger. Equal numbers of Go and No-Go stimuli were altered in both size and color to be more visually salient (SAL). Participants were asked to respond in the same manner to SAL stimuli as the regular, non-modified stimuli (REG). Whole-head magnetoencephalography (MEG) data were recorded with Neuromag Vectorview (Elekta). The signal was spectrally decomposed for each trial using Morlet wavelets in the theta (4-7 Hz) and beta (15-25 Hz) frequency bands. Each participant's cortical surface was reconstructed from high-resolution anatomical MRIs and served to constrain the distributed minimum norm inverse estimates. Behaviorally, task accuracy was differentially affected by stimulus type where SAL stimuli improved accuracy on No-Go trials but hindered accuracy on Go trials. Similarly, SAL Go response times were slower than REG Go, reflecting attentional capture. Successful response inhibition elicited an event-related theta power increase, peaking at ~300 ms, in a bilateral network including the inferior frontal, anterior cingulate, and insular regions, and right lateral temporal cortex. Overall there was a greater theta band increase in response to SAL stimuli compared to their REG counterparts as well as increased theta power to No-Go compared to Go trials. However, the anterior cingulate, inferior frontal, and lateral temporal regions showed particular sensitivity such that the theta power was the greatest to SAL No-Go trials. Though the activation in the anterior cingulate and inferior frontal region was bilateral, the theta power

increase estimated to the lateral temporal cortex was strongly right-lateralized. As expected, the event-related decrease in beta power was especially marked over the left motor regions subserving right-hand response preparation and execution. The beta band decrease was greater on SAL Go trials, indicating that the attentional capture affects motor output during downstream processing. Taken together, these data suggest that attentional capture plays a crucial role in informing and invoking the response inhibition system in an interactive manner.

Disclosures: **J.P. Happer:** None. **L.C. Wagner:** None. **L.E. Beaton:** None. **B.Q. Rosen:** None. **K. Marinkovic:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH/NS085543

Title: Surprising events interrupt visuospatial working memory

Authors: ***R. D. FINZI**[1], F. MARINI[1], B. R. POSTLE[2], A. ARON[1];
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Abstract: When a surprising event, such as a car honking, occurs in daily life, we often find that our ongoing train of thought is interrupted and consequently forgotten. In the lab, this situation has been modeled using a task of verbal working memory (WM) that is disruptable by surprising events. Using this paradigm, it has been shown that the disruption of verbal WM relates to the same fronto-basal ganglia mechanism that is thought to underlie the outright stopping of action (Wessel et al., 2016). While this provides new insights into the relationship between surprising events, WM, and motor inhibitory control, and could help to explain one form of distractibility, is it unknown whether the effect was due to the fact that verbal WM uses auditory and speech-based mechanisms, which may be especially disruptable by motor inhibitory control, and if the effect is thus specific to verbal WM. Moreover, the disruption effect observed in the previous study was short lasting; therefore whether the disruption effect can be extended across time remains to be established. Here we devised a visuospatial retro-cue paradigm. Participants were shown a number of square stimuli, based on an estimate of Cowan's *k* for each participant, in succession (each square was in a different position on the screen) and were then probed to recall the location of one of the squares. The probe consisted of a number cue indicating to participants which square of the sequence they would need to remember. Prior to presentation of the probe, a

sound was played. In Experiment 1 ($N = 16$), the sound was either a standard 600 Hz sine-wave tone (80% of trials) or a novel (a unique birdsong segment, 20% of trials), each played for 200 ms. While the proportion of novel trials was the same as in Wessel et al., 2016, nearly twice as many trials were used overall. We found a significant effect of surprise on visuospatial WM (as measured by the distance from the response location to the centroid of the target square) with decreased response precision after novel versus standard tones ($t(15) = 2.98$, $p = .009$, $d = 0.46$). We then replicated this effect in Experiment 2 ($N = 16$, $t(15) = 5.00$, $p < .001$, $d = 0.66$), including two types of surprising events: unique birdsong segments and longer duration sine-wave tones (500 ms), extending our findings to a different type of auditory novel. Thus, surprise impacts not only verbal WM (as in our earlier studies), but also visuospatial WM. Additionally, we show that the effect of surprise on cognition persists twice as long as previously shown, and that multiple types of novel events can produce the effect. This experimental paradigm is a good platform for further investigation of the neural correlates of the effect of surprise on WM.

Disclosures: **R.D. Finzi:** None. **F. Marini:** None. **B.R. Postle:** None. **A. Aron:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant K99-R00 MH096801

Title: Cognitive control networks route task information to other networks via intrinsic functional connectivity pathways

Authors: ***T. ITO**, D. H. SCHULTZ, L. I. SOLOMYAK, R. H. CHEN, R. D. MILL, M. W. COLE;
Ctr. for Mol. and Behavioral Neurosci., Rutgers Univ., Newark, NJ

Abstract: Functional connectivity (FC) patterns during rest and task are highly similar, suggesting an underlying intrinsic FC architecture shared between these states (Cole et al., 2014). Despite this, recent studies have provided evidence that cognitive control networks flexibly represent a dynamic range of task information through activity patterns and widespread task-evoked FC changes (Waskom et al., 2014; Cole et al., 2013). Here, we tested the hypothesis that intrinsic FC pathways carry the flow of task activity from cognitive control networks to content-related (e.g., visual and motor) networks to help implement task demands. We collected resting-state and task functional magnetic resonance imaging (fMRI) data while subjects performed a

complex cognitive paradigm involving the learning and implementation of multiple task rules (C-PRO, a variant of the paradigm from Cole et al., 2010). Using a recently developed method that linearly maps activity flow between regions via FC modulation, we extracted trial-by-trial task-rule activity patterns and mapped activity flow between networks. To test the information content of task-rule activity in the predicted network, we trained a linear classifier on predicted data while testing the classifier on the network's real data. We found that task-rule activity patterns generated in cognitive control networks could predict activity in content-related networks via activity flow for each of the three rule types (logic, sensory, and motor). Furthermore, network-to-network activity flow mappings were specific to the predicted network's functional relevance to the decoded rule type (e.g., motor network mappings for motor rules). To validate the computational principles underlying this approach, we constructed an abstract neural network model organized with distinct network communities to verify whether task activations in specific networks could be predicted based on network topology. Indeed, we found that intrinsic functional connectivity architectures could successfully predict the channels of activity flow. These findings suggest two conclusions: (1) Intrinsic FC reflects an underlying architecture that carries task information in the form of activity flow (the spread of cognitive task activation amplitudes), as validated through both empirical fMRI data and computational network models; (2) Flexible representations of task activity patterns in cognitive control networks contain information to downstream activity patterns in content-related networks.

Disclosures: **T. Ito:** None. **D.H. Schultz:** None. **L.I. Solomyak:** None. **R.H. Chen:** None. **R.D. Mill:** None. **M.W. Cole:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: 5R01MH49883-05

1P50MH106438-01

Title: Exploring the relationship between psychopathic traits and cognitive control performance in the context of reward

Authors: ***A. M. HOWELL**[1], T. SALO[2], P. K. PATEL[1], S. R. ASHBY[3], T. A. NIENDAM[1], T. A. LESH[1], C. S. CARTER[1];

[1]UC-Davis Imaging Res. Ctr., Sacramento, CA; [2]Florida Intl. Univ., Miami, FL; [3]Univ. of Oregon, Eugene, OR

Abstract: Although psychopathic traits (PT) are most often linked to emotional deficits, recent literature has shown PT are also linked to abnormal brain activation during cognitive control and reward processing tasks. However, the relationship between PT and cognitive control in the context of reward has not been studied extensively. We sought to explore the relationship between level of PT and engagement of cognitive control and reward networks. Specifically, we hypothesized that higher PT would relate to decreased activation in cognitive control networks and increased activation in reward systems.

Twenty-six healthy control participants completed the Incentivized Control Engagement Task (ICE-T), an incentivized version of a delayed match-to-sample task that separates reward anticipation and reward consumption in the context of cognitive control during fMRI. Participants also completed the Levenson Self-Report Psychopathy Scale (LSRPS) to determine level of psychopathy traits. Whole-brain regression analyses examined the relationship between LSRPS scores and BOLD activity during high versus low cognitive control trials as well as reward versus neutral trials. Whole-brain analyses were height thresholded at $p<.005$ and cluster-corrected at FWE $p<.05$.

LSRPS scores did not relate to participant's ability to perform more accurately or rapidly during high versus low cognitive control or rewarding versus neutral trials. Neuroimaging data indicated that higher LSRPS scores were associated with decreased BOLD during trials requiring higher cognitive control in the DLPFC, inferior parietal cortex, temporal lobe, somatosensory cortex, and cerebellum. While LSRPS did not show a significant relationship between BOLD and reward anticipation, significantly increased activity during the consummatory phase of reward compared to neutral trials was identified in DLPFC, ventral striatum, inferior parietal cortex, anterior cingulate cortex, thalamus, and supplementary motor cortex.

Neuroimaging data showed global cognitive control deficits and increased reward sensitivity in relationship to PT. Furthermore, people with increased PT engaged their attention and salience networks more during reward versus neutral trials. We hope to expand these results with further analyses differentiating the effects of affective/interpersonal (F1) and antisocial/lifestyle (F2) traits and compare individuals with unique combinations of F1 and F2 traits. Additionally, we hope to further assess the interaction of cognitive control and reward performance in these individuals.

Disclosures: **A.M. Howell:** None. **T. Salo:** None. **P.K. Patel:** None. **S.R. Ashby:** None. **T.A. Niendam:** None. **T.A. Lesh:** None. **C.S. Carter:** None.

Poster

647. Behavioral Demonstrations of Executive Function

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Program#/Poster#: 647.10/MMM19

Topic: H.02. Human Cognition and Behavior

Title: The importance of sleep quality on baseline concussion neurocognitive testing in collegiate student-athletes

Authors: *E. E. HALL[1], K. WARREN[1], L. STANDARD[1], R. HALLMAN[1], K. PATEL[2], C. J. KETCHAM[1];
[1]Exercise Sci., [2]Sports Med., Elon Universtiy, Elon, NC

Abstract: Baseline neurocognitive tests are a key component to concussion management protocols and are useful in helping make return-to-play (RTP) decisions. Previous research has shown that the amount of sleep that one has prior to neurocognitive testing may impact performance. However, the influence of sleep quality is less known. The purpose of this study was to examine the relationships between the amount of sleep and sleep quality on baseline neurocognitive performance. Ninety-seven NCAA Division I student-athletes completed the Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT^{TM}) test and the Pittsburg Sleep Quality Index questionnaire (PSQI) as part of baseline concussion testing. PSQI measures sleep quality based on responses to questions based on the past month. Number of hours of sleep the night before was reported into ImPACT. Of the 97 participants, 20 of the participants were considered poor sleepers based on the PSQI. MANOVA revealed that those considered poor sleepers had slower reaction time ($p = .031$) and higher total symptom score ($p < .01$). Based on the amount of sleep the night before, participants were classified as short (< 7 hours; $n = 26$); intermediate (7 to 9 hours; $n = 44$); and long (≥9 hours; $n = 4$). MANOVA on amount of sleep revealed no significant differences on scores on the ImPACT. These findings suggest that the amount of sleep the night before neurocognitive testing may not be as important as the sleep quality that they usually get the previous month. These results suggest that sleep and sleep quality may have an influence on neurocognitive performance and should be considered when making RTP decisions.

Disclosures: E.E. Hall: None. **K. Warren:** None. **L. Standard:** None. **R. Hallman:** None. **K. Patel:** None. **C.J. Ketcham:** None.

Poster

647. Behavioral Demonstrations of Executive Function

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Program#/Poster#: 647.11/MMM20

Topic: H.02. Human Cognition and Behavior

Support: University of Guanajuato

Title: Executive functions correlated with Body Mass Index in overweight middle aged women

Authors: ***M. SOLIS-ORTIZ**[1], L. MORADO-CRESPO[2], S. A. TREJO-BAHENA[2], L. KALA[3], M. L. GUTIERREZ-MUÑOZ[4];
[2]Ciencias Medicas, [1]Inst. Invest Med, Univ. Guanajuato, Leon, Mexico; [3]Medicina y Nutricion, Univ. de Guanajuato, Leon, Mexico; [4]Facultad de Psicologia, Univ. Autonoma de Nuevo Leon, Monterrey, Mexico, Nuevo Leon, Mexico

Abstract: The impacts of excess Body Mass Index (BMI) on physical health are well known and widely studied, but little is known about the consequences of being overweight or obese on cognitive function. The aim of this study was to correlate the BMI with executive functions in overweight middle-aged women. Seventy overweight middle-aged women between 48 and 64 years of age who are otherwise healthy participated in the study. Demographic and anthropometric variables were evaluated. Four standard neuropsychological tests were applied to assess executive functions, sustained attention, selective attention, and verbal fluency. The mean BMI of the participants was 29.35 and was negatively correlated with categories reached ($r = -0.41$, $p = 0.007$) and positively correlated with the number of errors ($r = 0.43$, $p = 0.005$) of executive function test. Scores of other neuropsychological tests applied showed no correlation with BMI. These findings suggest that BMI impacts the executive functions in overweight women who do not present any signs of disease.

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Poster

647. Behavioral Demonstrations of Executive Function

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Program#/Poster#: 647.12/MMM21

Topic: H.02. Human Cognition and Behavior

Title: Feasibility of at-home iPad cognitive testing

Authors: *N. NG, E. PETERSON;
Lumos Labs, Inc, San Francisco, CA

Abstract: The ability to monitor cognitive functioning across the lifespan accurately and remotely remains an essential method for understanding normal, healthy aging versus cognitive impairment and decline. Mobile, computerized neurocognitive assessments allow for less burdensome and more frequent administrations with minimal learning effects, which could potentially result in more accurate measures of an individual's performance in his natural environment and increase the reliability and the sensitivity of these assessments to cognitive change over time. For researchers, mobile assessments can aid in recruiting and running large-scale studies in a more cost-effective manner. In the clinical world, advantages of mobile cognitive assessments may enhance clinicians' ability to screen and longitudinally monitor patients' cognitive performance and with the hope of detecting decline or inconsistencies earlier. Additionally, mobile assessments make it substantially easier and cheaper to collect large samples of normative data for use in understanding how to interpret performance on mobile assessments.

While the capability of remotely administering cognitive assessments decreases burden of having a trained psychometrician or clinician present, development of these tools requires a balance between an easily understood self-guided experience and the psychometric requirements of good cognitive assessments. The mobile NeuroCognitive Performance Test (mNCPT; Lumos Labs, Inc.) is a mobile application and platform developed to remotely assess cognitive abilities in adults ages 18-90. The mNCPT is available for download on iPad and for this study was offered to a set of individuals who use Lumosity on their iPads. Individuals were guided through a battery of subtests to measure cognitive abilities, including working memory, attention and reasoning, and had the option of providing additional demographic information about themselves afterwards. To investigate the reliability of the mNCPT, a set of those who completed a first battery were asked to come back on a subsequent day to complete the same battery a second time. In this poster, we explore psychometric properties of the mNCPT and provide evidence for the feasibility and adequate reliability (average $r > 0.50$) of the mNCPT as a remote, unsupervised measure of cognitive performance.

Disclosures: **N. Ng:** A. Employment/Salary (full or part-time): Lumos Labs. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs. **E. Peterson:** A. Employment/Salary (full or part-time): Lumos Labs. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lumos Labs.

Poster

647. Behavioral Demonstrations of Executive Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 647.13/MMM22

Topic: H.02. Human Cognition and Behavior

Title: A novel design to dissociate experience- and expectation-based hierarchical control in task switching.

Authors: *C. LIU[1,2], N. YEUNG[2];
[1]Exptl. Psychology, Oxford, United Kingdom; [2]Dept. of Exptl. Psychology, Oxford, United Kingdom

Abstract: In both psychology and neuroscience, goal selection is held to be crucial to adaptive behavior. However, goals can be defined at multiple levels of abstraction: the same action can be treated as both taking a sip of coffee and refreshing oneself to work. Our work aims to study task-switching processes under these multiple levels of control. To date, some hints have suggested that hierarchical task organization might operate in task switching, in particular from studies showing that switching efficiency varies with the global probability of task switches (e.g., Monsell & Mizon, 2006). This finding suggests that contextual control information (about switch frequency) influences lower levels of control (guiding selection of specific tasks). However, an alternative interpretation of these results is that they reflect practice effects, with greater experience of switches improving their performance. To study multiple levels of top-down control, deconfounded from effects of experience, we developed a novel design to tease out pure top-down influences experience-driven adjustment.
Healthy human participants performed a standard task-switching paradigm in short blocks of trials. Importantly, participants' belief about switch probability in each block was manipulated through explicit instruction: Switch frequency instruction varied across blocks (frequent vs. rare switch) but objective frequency was matched for a subset of blocks (labeled as Fake Frequent and Fake Rare). Across these conditions, any difference in switching performance can be attributed exclusively to instruction and corresponding high-level control. Study 1 demonstrated behaviorally that instruction played a role above experience as evidenced by increased switch

costs in the Fake Rare vs. Fake Frequent condition. Study 2 manipulated reward to demonstrate that this effect was motivation-dependent. Study 3 used EEG methods to characterize the level at which instruction affected processing. We measured lateralized motor potentials to assess whether switch expectancy directly affects motor preparation for a task. On the other hand, expectancy could engage with attention system by strategically changing attentional scope as reflected in EEG alpha power. We found effects of instruction only on alpha power (Frequent Switch > Rare Switch). The results suggest that expectancy prompts the adoption of distinct control modes across blocks but not motor preparation of individual trial level.
Overall, we demonstrated that expectations uniquely contribute to adjustments in cognitive control in a task switching through hierarchical control.

Disclosures: **C. Liu:** None. **N. Yeung:** None.

Poster

647. Behavioral Demonstrations of Executive Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 647.14/MMM23

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant - R01

Title: Global motor suppression following action errors is related to post-error slowing and also to error-related interference

Authors: ***C. M. LEWIS**[1], A. R. ARON[2], J. R. WESSEL[3];
[1]Psychology, [2]Psychology/Neuroscience, Univ. of California San Diego, La Jolla, CA;
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Abstract: Many studies of performance-monitoring propose that after action errors, motor execution is slowed down to avoid further errors (post-error slowing, PES). However, a major challenge to these models comes from studies showing that when time to adapt is short, PES increases while post-error accuracy decreases. Here, we propose that this happens because a mechanism for global motor suppression is invoked to effect PES, which, however, negatively affects accuracy on the next trial. In 4 experiments using a foot-pedal Simon task with a short inter-trial interval, we tested whether error commission elicits global motor suppression by quantifying motor-evoked potentials (MEPs, elicited by transcranial magnetic stimulation) in task-unrelated hand muscles. Hand-MEPs were reduced at 250-300ms following error commission, indicating global motor suppression. We then tested whether the degree of this global motor suppression related to PES. Indeed, participants with greater motor suppression

showed increased PES. However, global motor suppression was negatively correlated with post-error accuracy: in all experiments, accuracy was decreased following action errors, and participants with stronger global motor suppression showed greater post-error accuracy deficits. We propose that following error commission, when time to adapt is short, a neural mechanism for global motor inhibition is invoked to effect PES. This global inhibition system may also impact cognition, affecting current task-set, and impacting subsequent behavior.

Disclosures: **C.M. Lewis:** None. **A.R. Aron:** None. **J.R. Wessel:** None.

Poster

647. Behavioral Demonstrations of Executive Function

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Topic: H.02. Human Cognition and Behavior

Support: UABC Grant 3607

PROMEP Grant UABC-PTC-486

Title: Executive functioning, academic self-efficacy, motivation, age and prior academic performance predict academic performance in university students

Authors: ***M. L. GARCÍA-GOMAR**, A. J. NEGRETE-CORTES, S. VALDEZ-HERNANDEZ, B. E. JIMENEZ-HIGUERA, D. I. ORTIZ CORTEZ, A. MONTOYA-VAZQUEZ;
Escuela de Ciencias de la Salud, Univ. Autónoma Del Estado De Baja California, Tijuana, Baja California, Mexico

Abstract: Objective: To study the relationship between executive functioning (EF), academic self-efficacy (SE), motivation, study habits and academic performance (AP) in university students. Methods: 75 students of health sciences were included in the study. EF was assessed with Modified Wisconsin Card Sorting Test (M-WCST) (Nelson, 1976), Verbal Fluency Test, Stroop Color-Word Interference Test, Symbol Digit Modalities Test (SDMT) (Smith, 1982) and Trail Making Test (TMT). Students were also assessed with Barrat Impulsivity Scale (Patton, Standford y Barrat, 1995), Academic Self-efficacy Scale (Blanco et al., 2011) and Inventory of Study Habits (Fernández-Pozar, 2014). GPA was considered as a measure of academic performance. Results: The students included in the study were 50 women and 25 men, with an average of 18.8±0.89 years old. All enrolled between first and second semester, 9.3% were medical students, 22.6% dentistry students, 18.6% nursing students and 49.3% psychology

students. Students had a GPA average of 83.2±11. Regarding EF assessment, low scores were found in EF tests. The students had an average of 4.5±1.9 categories in M-WCST (15th percentile); 5.9±6.6 perseverative errors (30th percentile); 6.8±7.6 Stroop interference score (40th percentile); 12.3±4.2 verbal fluency score (letter S) (20th percentile). The best fit linear regression model explained 37% of the variance in AP (p=0.00087) and included: prior AP (beta=0.3, p=0.02); Stroop interference score (beta=0.023, p=0.04); SE (beta=0.21, p=0.13); age (beta-0.21, p=0.05); motivation (beta=-0.20, p=0.10); communication factor in SE (beta=0.17, p=0.17); perseverative errors in M-WCST (beta=-0.16, p=0.14) and verbal fluency (beta=-0.13, p=0.24). Conclusions: The results support that prior AP, inhibition, SE, age, motivation, mental shifting and verbal fluency predict AP. Our results are in contrast with other studies in university population that found no relationship between EF and AP (Barceló-Martínez, Lewis Harb & Moreno-Torres, 2006; Vergara-Mesa, 2011) but agree with studies that found a positive relationship between EF and AP in children and adolescents (Bull, Espy & Wiebe, 2007; St Claire-Thomson & Gathercole, 2006; Latzman, Elkovitch, Young, Lee & Clarck, 2010). Our results showed that inhibition, mental shifting and verbal fluency are the EF components most related with AP, in contrast to other studies which found that working memory is the most related EF component (Da Costa Leite, 2013; Thorell, Veleiro, Siu, Mohammadi, 2013). It is important to increase the sample size, to include a working memory test and psychophysiological measures to confirm the results.

Disclosures: M.L. García-Gomar: None. **A.J. Negrete-Cortes:** None. **S. Valdez-Hernandez:** None. **B.E. Jimenez-Higuera:** None. **D.I. Ortiz Cortez:** None. **A. Montoya-Vazquez:** None.

Poster

647. Behavioral Demonstrations of Executive Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 647.16/MMM25

Topic: H.02. Human Cognition and Behavior

Title: Network signatures of flexible cognitive control are reflected in oscillatory MEG source connectivity

Authors: *R. D. MILL[1], A. BAGIC[2], W. SCHNEIDER[2], M. W. COLE[1];
[1]Ctr. for Mol. and Behavioral Neurosci., Rutgers Univ. Newark, Newark, NJ; [2]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The ability to flexibly adjust thoughts and behavior across diverse task domains is a fundamental feature of cognitive control. Previous fMRI research in humans has linked such control processing to the cross-domain engagement of cognitive control brain networks, which

alter their patterns of functional connectivity with domain-specific networks in response to ongoing task demands (Cole et al., 2013; *Nat Neuro*). However, fMRI imaging is limited in its capacity to resolve the fine-grained temporal signatures underlying flexible control network recruitment. Furthermore, prior functional connectivity studies using higher temporal resolution imaging methods (e.g. M/EEG) have either been confined to network analyses at the raw sensor level (rather than the underlying neural source space) or have primarily focused on the resting state (which lacks explicit experimenter control over ongoing task demands). Hence, subjects in the current study underwent MEG scanning whilst performing the permuted rule operations task (PRO) - a paradigm that iterates through 4 categories of each of 3 rule domains (4 logic x 4 sensory x 4 response rules) to present a total of 64 unique task states (Cole et al., 2010; *J Neuro*). Using adaptive linear beamforming methods of source localisation and individualized head models constrained to subjects' anatomical MRIs, we reconstructed spatio-temporally precise source activation timeseries from 264 brain regions specified from a functional atlas (Power et al., 2011; *Neuron*). The source timeseries were resolved to separate frequency bands (via band-pass filtering and the Hilbert transform), and corrected for source mixing/spread artifacts by the removal of spurious 'zero-lag' phase relationships between source pairs (Hipp et al., 2012; *Nat Neuro*). The resultant band-limited, phase-orthogonalized source power envelopes were correlated on a pairwise basis to estimate functional connectivity during performance of the PRO paradigm. Consistent with previous fMRI findings, graph theoretic analyses applied to the MEG source connectivity estimates identified flexible patterns of network engagement across multiple task states that were specific to cognitive control networks. Our results go further in segregating these flexible network signatures as a function of frequency band and the specificity of task processing (e.g. by contrasting connectivity during 'initial encoding' versus 'active performance' of the different PRO tasks). The findings provide insight into the dynamic, oscillatory brain network processes that underpin controlled cognitive processing across multiple task domains.

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Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 648.01/MMM26

Topic: H.02. Human Cognition and Behavior

Support: ARC DE140100350

Title: Predicting outcome and timing of upcoming decisions using multivariate pattern analysis for event-related potentials

Authors: *S. BODE[1], H. KEAGE[2], D. FEUERRIEGEL[2], M. E. R. NICHOLLS[3], O. CHURCHES[3];
[1]The Univ. of Melbourne, Parkville, Australia; [2]Sch. of Psychology, Social Work and Social Policy, Univ. of South Australia, Adelaide, Australia; [3]Sch. of Psychology, Flinders Univ., Adelaide, Australia

Abstract: Over the past decade, multivariate pattern classification analysis (MVPA) has become a valuable tool for the analysis of human neuroimaging data. Recently, these methods have been successfully applied to spatially distributed patterns of event-related potential (ERP) data to predict upcoming decisions during the early stages of information processing (Bode et al., 2012, *J Neurosci*; Bode & Stahl, 2014, *Biol Psychol*). One important question that remains is whether this early information directly informs subsequent decision behaviour. The present study therefore investigated the extent to which decodable decision-relevant information predicted the timing of behavioural responses. ERP data was recorded from 12 participants (18-37yrs, 6 female) during a series of simple identity decisions, whereby two consecutively presented letters were shown in 12 different rotation angle conditions (0°-330°, in steps of 30°). The electroencephalogram was recorded at 1000Hz using a Quickcap (Neuroscan) system with 64 silver/silver-chloride electrodes. After standard pre-processing, decision outcomes were predicted from spatio-temporal activity patterns in 10ms analysis time windows, which were moved through each trial. We identified the first time window from which decisions could be predicted at ~200-260ms after the presentation of the probe stimulus, depending on rotation angle condition. This information preceded the average response time (RT) by ~200-250ms. To estimate the extent to which decision-information from these early time windows was related to RT, the *change* in mean classification accuracy was correlated with the *change* in mean RT for each rotation angle condition when excluding each participant once. On average, the amount of decision-relevant information that correlated significantly with RT started at 260ms after the probe stimulus, and peaked at ~400ms, which corresponded to the beginning of the response period (mean $r = -.45$; $p < .05$ Bonferroni-corrected). This suggests that when more information was represented in brain activity patterns during early time windows, faster decisions were made. These results provide strong evidence that MVPA for ERPs can reveal the accumulation of information in the brain that is directly relevant for upcoming behaviour.

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Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

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Program#/Poster#: 648.02/MMM27

Topic: H.02. Human Cognition and Behavior

Support: Swartz Foundation Gift

Title: Human brain dynamics supporting emergence of sudden insight

Authors: *Y. J. WU[1], S. MAKEIG[2];
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Abstract: Sudden insight during problem solving results from enlarging or restructuring a problem solution space, allowing a previously inaccessible solution to emerge into the participant's imagination, producing an 'Aha!' experience. As the subjective suddenness of such moments suggest their precipitation by a transient brain dynamic event, much research on insight during human problem solving has focused on the second immediately preceding a participant's signal (by button press) that they have discovered a solution. In the past decade, however, a number of studies have indicated a direct relationship between brain processes occurring in earlier stages of problem solving and the subsequent 'Aha!' experience. To examine brain activities during the full time course of a solution search process, we recorded high-density EEG data as healthy adults solved 'Wheel-of-Fortune' style language puzzles. Each trial began with a series of dashes and blank spaces on a screen indicating the positions of letters in a common phrase or idiom. At randomly jittered intervals (1.5-3s), single letters in the phrase, randomly selected by the game application, replaced the dashes. Participants pressed a button as soon as they could guess the full phrase. After a cue, they spoke their solutions aloud, allowing the experimenter to check accuracy. The participants then rated the degree of sudden insight they had experienced when reaching the solution. Correct solutions were reached after presentations of two to twelve letter clues, and were later rewarded with a monetary bonus proportional to solution speed. Pre-cleaned 256-channel EEG data were decomposed into underlying source contributions by adaptive mixture independent component analysis (AMICA). Brain-based independent component (IC) scalp maps were modeled as projections of single equivalent dipoles, and event-related spectral perturbations (ERSPs) were computed for each such IC in 1.5-s epochs following each letter clue presentation. Measure Projection Analysis (MPA) identified a subspace of model brain voxels exhibiting consistent cue-onset ERSP patterns across ICs with nearby equivalent dipoles. This consistency voxel subspace was further divided into distinct domains by Affinity clustering on voxel-centered mean ERSP differences. Results revealed spectral power changes in right hemisphere cortical field activity beginning at least three letter presentations before participants articulated the correct solution. This result implies that problem solving in this task is a prolonged process with distinct brain dynamic pre-conditions.

Disclosures: Y.J. Wu: None. **S. Makeig:** None.

Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

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Program#/Poster#: 648.03/MMM28

Topic: H.02. Human Cognition and Behavior

Support: NIH grant R01DA038063

Title: Normative foundations for value normalization in choice behavior: balancing outcomes with entropic costs

Authors: *C. K. STEVERSON, A. BRANDENBURGER, P. GLIMCHER;
NYU, New York, NY

Abstract: Barlow (1961) argued that meaningful study of neural mechanisms requires an understanding of their functional importance. In the case of the divisive normalization model of visual systems (Carandini and Heeger 2012), it has been argued that the functional importance of normalization comes through the efficient coding of stimuli (Schwartz and Simoncelli 2001, Lyu and Simoncelli 2009). More recently, the normalization mechanism has also been observed in value representation and choice behavior. Value normalization has successfully explained neuronal activity in primates (Louie et al. 2011) and experimental choice behavior in humans (Louie et al. 2013, Web et al. 2014). However, stimulus encoding is not the purpose of choice, so that efficient coding cannot explain the functional importance of normalization in choice behavior. We propose instead that value normalization plays a role in bringing about efficient choice. Specifically, we show that any behavior arising from a class of value normalization models can be rationalized as maximizing the expected value of choice minus the costs of decision making. We also establish that our class of value normalization models covers all the behaviors that can be rationalized by our model of costly decision making. We model the costs of decision making via the Shannon entropy of the choice probabilities, which is a measure of the degree of stochasticity in choice. Less stochasticity in choice requires a larger reduction in entropy and is therefore more costly. In addition, we scale the costs of decision making via a set-dependent factor that allows choice sets to vary in how hard it is to discriminate among the alternatives. For instance, choice sets might vary in the similarity or salience of the alternatives they contain. We connect our normalization model to the classic Luce model of stochastic choice which is popular in the field of economics. We show how a relaxation of the condition called independence from irrelevant alternatives, which is known to characterize the Luce model, leads to normalization. Our work provides a normative foundation for value normalization by deriving it as the class of optimal solutions to a problem of costly decision making. This normative foundation lends support to the possibility that the functional importance of value normalization lies in bringing about efficient choice behavior.

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Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

Location: Halls B-H

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Program#/Poster#: 648.04/MMM29

Topic: G.03. Emotion

Support: NSF EFRI MC3 1137237

Title: Uncovering the dynamical neural basis of human decision making system

Authors: ***P. SACRÉ**[1], S. SUBRAMANIAN[2], M. S. D. KERR[2], K. KAHN[3], J. GONZALEZ-MARTINEZ[4], M. A. JOHNSON[4], J. T. GALE[4], S. SARMA[1];
[1]Dept. of biomedical engineering, Johns Hopkins Univ., Baltimore, MD; [2]Univ. of Cambridge, Cambridge, United Kingdom; [3]Insight Data Sci., Palo Alto, CA; [4]Cleveland Clin., Cleveland, OH

Abstract: Decision-making links cognition to behavior and is a key driver of human personality, fundamental for survival, and essential for our ability to learn and adapt. It has been well established that humans make logical decisions where they maximize an expected reward, but this rationality is influenced by their emotional state. Psychiatric patients who have dysfunctional cognitive and emotional circuitry frequently have severe alterations in decision-making where emotion hijacks logic. Unfortunately, the function of relevant neural circuits in humans is largely uncharted at fine temporal scales, severely limiting the understanding of changes underlying disruption associated with age or psychiatric diseases. In this study, we localize neural populations, circuits, and their temporal patterns *on a millisecond scale* that are critically involved in human decision-making.
Twelve human subjects, implanted with multiple depth electrodes for clinical purposes, performed a gambling task while we recorded local field potential neural activity from deep and peripheral brain structures. The gambling task consisted of a game of "high card", where the subject bets high ($20) or low ($5) on whether her card will be higher than the computer's card. We posited that each subject's decision-making system consists of a feedforward model with the playing card as the input and betting behavior as the output (e.g. how she bets and how quickly she bets). The behavior and gambling outcome is a feedback control signal to the model, which updates the internal latent state of the feedforward model. For each subject, we estimated the latent state variable from binary bets (high versus low) using maximum likelihood methods. Specifically, we modeled the probability of betting high as a function of this internal state and the player's card, and the state updates on each trial as a function of observed quantities such as

the card value, expected reward, and variance of reward.
Further, neural correlates for the internal state trajectory and components that update the internal state are present in key limbic structures suggesting that the internal state is correlated to the subject's emotions. In particular, specific oscillations in brain structures, including amygdala, cingulate cortex, and the entorhinal complex are shown to influence betting behavior (what you bet and how quickly you make the bet) in a profound way. These findings provide new insight into how humans link their internal biases (e.g. emotions) to decisions.

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Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

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Medical Research Council Studentship

Max Planck Society

Title: Quality and quantity in value-based decision making

Authors: ***A. O. DE BERKER**, Z. KURTH-NELSON, R. B. RUTLEDGE, S. BESTMANN, R. DOLAN;
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Abstract: Quality and quantity are cardinal dimensions in value-based decision-making. To make effective decisions it is necessary to combine information about both in order to estimate an integrated value for available options. This calculation requires an interaction between quality and quantity, given that the value of one additional unit of a good depends fundamentally upon the unitary value of that good; an extra car is worth more than an extra pair of socks. Here we investigate how quality and quantity are represented in the brain, and how they interact to produce integrated value. In a behavioral experiment we first assessed individual value functions

for different gift cards (e.g., supermarket, book store) using Becker-DeGroot-Marschak auctions. We then used fMRI to identify the neural representation of quality (how desirable the brand of each gift card was), quantity (how much money was on each gift card), and the interaction between these two dimensions. Activity in lateral and inferior prefrontal and temporal cortices correlated with gift card quality, while activity in intra-parietal sulcus and surrounding parietal cortex reflected the quantity. A network comprising the cingulate cortex, bilateral hippocampus, and medial prefrontal cortex encoded an interaction between quality and quantity. Conjunction analyses revealed overlapping representations of quality, quantity, and their interaction in the anterior cingulate, suggesting that this region provides the substrate for the formation of an integrated value from quality and quantity.

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Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

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Program#/Poster#: 648.06/MMM31

Topic: H.02. Human Cognition and Behavior

Support: Duke Institute for Brain Sciences Research Incubator Award

Title: A quantitative meta-analysis of neuroimaging studies on counterfactual thinking

Authors: *F. DE BRIGARD[1], G. STEWART[1], N. PARIKH[1], N. SPRENG[2];
[1]Psychology, Duke Univ., Durham, NC; [2]Human Develop., Cornell Univ., Ithaca, NY

Abstract: Counterfactual thinking refers to our psychological capacity to entertain thoughts about alternative ways in which past events could have occurred but did not. In the past decade, cognitive neuroscientists have explored neural activations associated with counterfactual thinking either in the context of decision-making or in the context of mental simulation. However, the extent to which both kinds of counterfactual thinking tasks engage common brain regions remains unknown. To address this question, we present here a quantitative meta-analysis of neuroimaging studies of counterfactual thinking from both the decision-making and the mental simulation literatures. After a systematic search of the scientific literature, 18 neuroimaging studies involving counterfactual thinking tasks were identified. Next, a coordinate-based meta-analysis using Activation Likelihood Estimation (ALE) was conducted. This analysis revealed seven clusters reliably activated during counterfactual thinking tasks: right lateral parietal cortex (BA39), left superior prefrontal cortex (BA 8), bilateral globus pallidus in the

basal ganglia, right hippocampus, inferior prefrontal cortex (BA 47), anterior cingulate cortex, and left precentral gyrus (BA 9). These results provide reliable and robust estimates of the neural correlates of counterfactual thinking. In addition, we believe these results add to growing evidence suggesting that the hippocampus plays a critical role during mental simulation and decision-making tasks involving thoughts about unactualized events. Finally, these results suggest further avenues to research interactions and differential contributions of basal ganglia, parietal and pre-frontal cortices during counterfactual thinking tasks.

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Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

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Topic: H.02. Human Cognition and Behavior

Support: EU Grant LSHM-CT- 2007-037286

DFG Grant PE1627/3-1

Title: The role of reward immediacy in temporal discounting

Authors: ***U. BROMBERG**, J. PETERS, C. BUCHEL;
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Abstract: Temporal discounting tasks require agents to engage in a trade-off between value and time, while making choices between smaller but sooner and larger but later rewards. The degree of discounting of reward-values that are burdened with a timely delay, varies considerably among individuals, giving rise to subject-specific values of the rewards. Neural correlates of subjective values in discounting can be characterized by parametric value representations (Kable & Glimcher, 2007, 2010; Miedl et al., 2012; Peters & Büchel, 2009). It has been shown that the availability of immediate rewards increase responses in brain regions involved in reward value coding (McClure et al., 2004; McClure et al., 2007; Luo et al., 2009), but it is not yet known whether parametric value representations are modulated by the immediacy of reward. Here we investigate the impact of immediate rewards on parametric value representation as well as related functional connectivity in meso-limbic circuits, that are possibly modulated by immediate rewards (Ousdal et al., 2012; Ambroggi et al., 2008). Our sample of young adults (n = 55, age 18-20 years, 30 females) performed a discounting task that included two conditions: choices with immediate rewards (now-trials = NT) and choices without immediate rewards (not-now-trials =

NNT). We tested our results in an age- and sex-matched independent replication sample (n = 55, age 18-20 years, 30 females). We report an impact of immediacy on neural subjective value representation with stronger activation in the medial and bilateral OFC for NT as compared to NNT. No differences in activation was found for NNT as compared to NT. We also report results from psycho-physiological analyses. Our results confirm a previously reported impact of immediacy on neural reward processing in temporal discounting. Specifically we show an impact on neural parametric value representation of discounting behavior. The results are discussed in relation to several discounting relevant measures such as personality and substance use.

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Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

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Medical Research Council MC_UU_12024/1

Title: Neural mechanisms underlying speed-accuracy adjustments in the subthalamic nucleus.

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Abstract: Neurobiological models of decision-making propose that the subthalamic nucleus (STN) plays a central role when humans weigh quick but possibly erroneous decisions against more accurate but slow decisions. However, to date there is no direct electrophysiological evidence linking STN activity to such trade-offs between speed and accuracy. To address this, we recorded STN local field potentials (LFP) from eleven Parkinson patients who had undergone STN deep brain stimulation surgery. During the recordings participants performed a task in

which they had to decide whether a cloud of moving dots appeared to move to the left or to the right. The percentage of dots moving coherently in the correct direction was either high (50%) or low (8%), and participants were instructed to respond as quickly or as accurately as possible indicated by a cue presented before the onset of the moving dots. In order to relate recorded neural activity to distinct aspects of the decision-making process, we applied Bayesian hierarchical drift diffusion modelling (HDDM). HDDM computes the rate at which evidence accumulates over time (drift rate), the level of evidence at which participants respond (decision threshold) as well as the non-decision time based on participants' behaviour. For all statistical analyses, significance thresholds were set to 0.05. As expected, subjects responded slower when they were instructed to focus on accuracy, not speed. In addition, participants were slower and less accurate during trials with low compared to high coherence. HDDM revealed that these behavioural changes were related to an increase in decision thresholds following cues instructing accuracy relative to speed and a decrease in drift rates in low compared to high coherence trials. Analysis of STN LFPs showed that instructing patients to speed up responses increased low-frequency oscillatory (LFO) activity (2-8 Hz) and induced a stronger initial decrease in beta activity (13-30 Hz) between the onset of moving dots and the response. Interestingly, these two neural processes were not statistically related to each other as indicated by correlation analyses. Including the pre-response changes in STN LFO- and beta activity as predictors in HDDM regression analyses revealed that increased LFO activity predicted increased decision thresholds only after accuracy but not speed instructions, while stronger decreases in beta activity predicted decreased decision thresholds irrespective of the instruction. These findings indicate that distinct processes in the STN mediate adjustments between fast and accurate decisions by modulating how much evidence subjects acquire before committing to a decision.

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Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

Location: Halls B-H

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Support: Virginia Tech

Wellcome Trust

Kane Family Foudation

NIH Grant RO1NS092701

Title: Dopamine and serotonin coordinate moment-to-moment encoding of behavioral control signals in humans.

Authors: *K. T. KISHIDA[1], R. MORAN[2], J. WHITE[2], E. LAWRENCE[2], T. LOHRENZ[2], I. SAEZ[3], A. LAXTON[4], M. R. WITCHER[4], S. B. TATTER[4], P. E. M. PHILLIPS[5], P. DAYAN[6], P. R. MONTAGUE[2];
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Abstract: The neurotransmitters dopamine and serotonin are important modulators of neural activity and synaptic plasticity. Much work has been performed in model organisms to elucidate how these systems influence behavior and learning. However, technological limitations have hindered investigation of how these systems coordinate in real-time in humans to control behavior and influence learning. Here, we report simultaneous real-time (10Hz) measurements of dopamine and serotonin fluctuations in the striatum of 25 humans engaged in a continuous decision-making task. These measurements were made using fast scan cyclic voltammetry on carbon fiber microelectrodes acutely implanted in the striatum of patients undergoing deep brain stimulation electrode implantation for the treatment of Parkinson's disease symptoms. Penalized regression and a substantial calibration data set were used to train models for estimating dopamine and serotonin levels from non-background subtracted voltammograms. We demonstrate that this approach produces models that: (1) accurately track dopamine and serotonin fluctuations for long periods (>30min), (2) discriminate dopamine, serotonin, and pH fluctuations, while (3) overcoming the effects of electrode fouling and background drift that have plagued previous approaches. We applied these models to cyclic voltammograms collected from patients playing a sequential investment game. Sub-second dopamine and serotonin fluctuations encoding changes in expectations and evaluative feedback; further, fluctuations in one trial were predictive of actions in the next trial. These methods promise unprecedented insight into dopamine and serotonin systems in human decision-making, health, and disease.

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Poster

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Support: US-German Collaboration: Computational and Neural Mechanisms of Inference over Decision-Structure

The Nakajima Foundation

Title: Neural correlates of temporal change point detection in the human brain

Authors: *R. ADACHI, S. SUZUKI, J. P. O'DOHERTY;
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Abstract: Detecting changes in the timing of event occurrence is prevalent across species. Toads detect changes in the pattern of ripples on the water's surface caused by the movement of potential prey while human stock traders detect changes in the arrival of transactions to figure out shifts in investor sentiment. In this experiment, we investigated the behavioral and neural underpinnings of temporal change point detection. In the task, subjects observed a sequence of images presented on the screen while being scanned in the fMRI scanner. The sequences contained abrupt changes in the rate of image presentation and the subjects reported perceived changes by the button press. Subjects' performance was significantly better than chance and we observed considerable heterogeneity in performance across subjects. To describe subject behavior quantitatively, we developed a frugal temporal averaging model, that compared the average rate of image presentation in a moving window defined over the recent past against the average rate estimated over a longer temporal interval. We compared the performance of the temporal average model to a more computationally expensive Bayesian online change point detection model inspired by Adams and MacKay (2007). Surprisingly, we found that the simple temporal average model captured the important aspects in the behavioral data as well as the Bayesian model despite the difference in their model complexity. We then correlated the time course of the decision variable predicted by the temporal average model against fMRI data. We found that the difference in the rate of image presentation exhibited a positive correlation with BOLD activity in a region of dorsomedial prefrontal cortex. Subsequent analyses will further explore the relationship between behavioral and neural data across subjects.

Disclosures: **R. Adachi:** None. **S. Suzuki:** None. **J.P. O'Doherty:** None.

Poster

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Program#/Poster#: 648.11/MMM36

Topic: H.02. Human Cognition and Behavior

Support: German Research Foundation (DFG) grant Wl 4094/2-1

Title: The effect of contextual novelty on monetary intertemporal choice

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Abstract: In intertemporal choice, humans often discount the value of delayed rewards, preferring immediate rewards. When the average reward rate (expected reward per time unit) increases, the cost of waiting for a future reward weighs more heavily, increasing temporal discounting. Tonic dopaminergic activity has been suggested to represent the average reward rate, and pharmacological enhancement of dopamine transmission increases discounting. Tonic dopamine is also increased in novel environments. In the present three-day fMRI study, we investigated whether exposure to novelty modulates subsequent intertemporal choice. On day 1, participants' discount rate was measured and they were familiarized with a set of images. On days 2 and 3, participants were first presented with familiar or novel images for 5 minutes. Subsequently, fMRI data were collected during an intertemporal choice task. Preliminary results (N = 10) suggest that the correlation between striatal activity and the subjective value of the chosen option is higher after exposure to novel compared to familiar stimuli, pointing to a possibility of novelty-associated modulation of intertemporal choice.

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Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

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Topic: H.02. Human Cognition and Behavior

Title: Predicting individual differences in impulsivity using resting-state fMRI and machine learning

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Abstract: The development of objective biomarkers for addictive disorders can innovate their prevention and treatment. Abundant evidence indicates that addictive disorders are characterized by neurocognitive manifestations of *impulsivity*, one of the most viable endophenotypic markers for addiction. A promising neuroimaging-based biomarker for addiction and related traits is resting-state functional magnetic resonance imaging (rs-fMRI) connectivity. Previous studies demonstrated its association with impulsivity (e.g., Davis et al., 2013) and rs-fMRI connectivity predicted the status of addiction dependence (e.g., Pariyadath et al., 2015). However, it remains unclear if individual differences in impulsivity can be accurately predicted by rs-fMRI connectivity.
To test if rs-fMRI connectivity can predict individual differences in impulsivity, we used 926 participants' rs-fMRI data provided by the Brain Genomics Superstruct Project (GSP). The Conn toolbox was used to preprocess and perform ROI-to-ROI connectivity analysis in each subject. We tested several machine learning algorithms to predict impulsivity (total scores of the Barratt Impulsiveness Scale) using ROI-to-ROI connectivity measures in new samples. Each machine learning algorithm was fitted on the same training set (617 participants' data) using 10-fold cross validation and we tested its out-of-sample prediction accuracy in the test set (309 participants' data). Our preliminary results suggest that rs-fMRI connectivity can predict impulsivity scores in new samples and it is our expectation that this line of work may shed light on the development of biomarkers that can be used to assess individuals' risk to addiction in clinical settings.
Acknowledgement: Data were provided [in part] by the Brain Genomics Superstruct Project of Harvard University and the Massachusetts General Hospital, (Principal Investigators: Randy Buckner, Joshua Roffman, and Jordan Smoller), with support from the Center for Brain Science Neuroinformatics Research Group, the Athinoula A. Martinos Center for Biomedical Imaging, and the Center for Human Genetic Research. 20 individual investigators at Harvard and MGH generously contributed data to the overall project.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIA K23AG043553

NIA R01AG022983

Title: Specific impairment in integrating quantitative features of intertemporal choice in Alzheimer's disease

Authors: ***A. J. BEAGLE**[1], J. H. KRAMER[2], M. HSU[3], A. S. KAYSER[2], K. A. WOOD[2], W. CHIONG[2];
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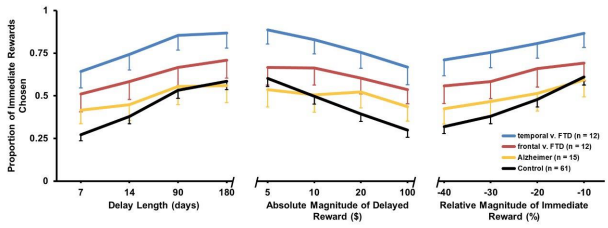
Abstract: It has been proposed that economic choice involves a two-stage process in which the subjective value of each option is computed, and then these values are compared to arrive at a decision (Padoa-Schioppa, Ann Rev Neurosci 2011). Such neuroeconomic models may help explain decision-making impairments observed in neurodegenerative diseases such as Alzheimer's disease (AD) and frontotemporal dementia (FTD). We investigated whether conditions targeting different anatomic systems would have dissociable effects on neural mechanisms of decision-making in intertemporal choice. We have reported that patients with temporal lobe-predominant FTD make a greater proportion of impulsive choices than controls, while patients with AD make a similar proportion of impulsive choices to controls (Chiong et al, Brain 2016). In this analysis, we studied participants' sensitivity to quantitative and temporal features of a hypothetical two-choice delay discounting task in which the delay length and the absolute and relative magnitudes of rewards were fully crossed. Controls responded to increases in delay length, decreases in the absolute magnitude of the delayed reward, and increases in the relative magnitude of the immediate reward by more frequently choosing the immediate reward. Patients with AD had diminished sensitivity to differences in delay length ($p < 0.01$) and the absolute ($p < 0.01$) and relative reward magnitude ($p < 0.01$), though their baseline tendency to choose an immediate reward did not differ ($p = 0.45$). Patients with temporal variant FTD did not differ from controls in their sensitivity to these features ($p = 0.45$, 0.93 & 0.07), but had a greater baseline tendency to choose an immediate reward ($p < 0.01$). Patients with frontal variant FTD ($n = 12$) demonstrated an intermediate phenotype. These results suggest that impaired decision-making in AD may result from failures to integrate quantitative features of available options in subjective valuation, while in the temporal variant of FTD it may result from a systematic bias towards impulsive choice that is independent of such integration.



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Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

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Topic: H.02. Human Cognition and Behavior

Title: Age differences in mutual information of electroencephalograms during value-based decision-making

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Abstract: The aim of this study was to evaluate age-related temporal differences in neural information transmission when young and older adults make value-based decisions. We used time-delayed cross mutual information (CMI) to measure linear and nonlinear dynamical coupling of EEG signals between electrodes. EEGs from 32 channels were recorded in 11 young (6/5 males/females; age = 24±1.9 yrs, mean±SD) and 11 older (3/8 males/females; age = 68±4.0 yrs) normal participants during a lottery choice task. All data were filtered with a band pass of 0.1-50 Hz. Participants decided to accept or reject stakes consisting of high, middle, and low levels of points magnitudes and probabilities of winning (or losing) the points. Focusing on central line electrodes (CZ, FZ), we computed CMI time courses affording onset, peak, and terminating times of waves of information transmission occurring after stimulus onset for each subject and then evaluated age differences in peak latencies. Older adults displayed more risky behavior with higher acceptance rates than the young for more costly low win probability conditions (high lose probability) (Young: 0.02±0.09; Old: 0.15±0.20 ; $p < .05$). We found significant age difference ($p<.001$) in the times of the first CMI peak such that signal in CZ was correlated with that in FZ between 88±18 - 228±54ms in the young group but between 181±60 - 354±87ms in the older group. Thus, it took less time for the first peak of neural information transmission in young than older adults. However, the effect of probability was trivial in the first peak suggesting that it reflected basic representational processes. Critically, both age and probability modulated onsets of subsequent second CMI peaks. Whereas there were no age differences during the high probability condition (310±62 - 472±77ms), the second CMI peak

times were earlier in younger than older adults in the middle (Young: 244±30 - 415±81ms; Old: 439±97 - 587±98ms) and low (Young: 240±56 - 400±95ms; Old: 385±36 - 609±88ms) probability conditions. Further, while the probability effect was not significant in the young group, the time of the second CMI peak in middle and low probability conditions were significantly later than that in the high probability condition in the older group (334±72 - 473±105ms; $p < .05$). Our findings suggest that risky behavior in older adults is associated with more temporally extended neural processing compared to younger adults. We further demonstrate that CMI between EEG electrodes captures psychologically and neurophysiologically meaningful aspects of human processing of value-based decisions.

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Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

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Topic: H.02. Human Cognition and Behavior

Support: NSERC DG

Title: Using rapid reaching to explore the value of information

Authors: *C. S. CHAPMAN[1,2], S. HO[2], J. SAWALHA[1], I. I. GILANI[2], N. J. WISPINSKI[3], J. K. BERTRAND[1], E. B. LAVOIE[1];
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Abstract: Is there a difference between knowing you will receive a reward and actually getting it? These two factors, here called information (info) and action-outcome (action), are usually intertwined, but may bias a decision in different ways. In the two reported experiments (E1 & E2) we teased apart how these two factors biased rapid reach decisions.
For both experiments, participants completed two phases. In the Value Learning (VL) phase, they learned to associate specific shapes with specific outcomes. In the Rapid Reaching (RR) phase, they were told to ignore the learned associations and hit one of two targets cued at reach onset. During VL trials, participants saw shapes at only a single target location. A first shape (info) came on the screen and served as a movement pre-cue. After a brief delay, an auditory go cue signaled that participants should reach for that shape. Upon movement onset, the first shape was replaced by a second shape (action). Participants had to successfully reach toward and hit the action target to receive reward points for that trial. In E1 info shapes predicted action shapes

100% of the time. One info-action pair produced reward 100% of the time and a second info-action pair produced reward 50% of the time. In E2 info shapes were not predictive of action shapes, but each action shape was still consistently paired with either 100% or 50% reward. During RR trials, participants were presented with two of the four VL shapes coincident with the go cue and had to rapidly initiate a movement. At movement onset one of the two shapes was selected (filled-in black) and participants had to rapidly reach to its location to receive a reward for that trial. Possible rewards for RR trials were constant and based only on rapid reach performance (e.g. shape was irrelevant).
We used functional regression techniques on RR hand trajectories to separately reveal the time evolving bias induced by two factors acquired during VL: information vs action and 100% reward vs 50% reward. In E1 we found the hand was significantly biased toward information targets and 100% reward targets. In E2, even though the information targets no longer predicted reward, we saw the same pattern of results. We hypothesize that info targets are valuable in part because they predict reward (E1) but more because they predict the upcoming action location (E1 & E2). This suggests that successfully completed actions are intrinsically and powerfully rewarding, and this reward signal plays an integral role in shaping learning and behavior.

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Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

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HHMI International Student Research

James S. McDonnell Foundation

Title: The speed-accuracy tradeoff regulates the rate of evidence accumulation, non-decision time, and decision boundary

Authors: *S. ITTHIPURIPAT[1], J. T. SERENCES[2];
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Abstract: Manipulating the speed-accuracy tradeoff (SAT) has a significant impact on the underlying cognitive processes that support decision-making. Multiple behavioral studies in human suggest that the SAT is best explained via changes in the amount of evidence required before making a decision (i.e. the *decision boundary*). However, a recent study using non-human primates found that neural responses recorded from the lateral intraparietal (LIP) area, which is thought to track evidence accumulation, reached the same threshold under different SAT regimes. Here, we tested the generality of this result in humans by examining how the SAT impacts the cento-parietal potential (CPP), which has been shown to track evidence accumulation in human subjects in a similar manner to LIP neurons in non-human primates. To do so, we recorded electroencephalography (EEG) from human subjects while they were performing a contrast discrimination task where either speed or accuracy was emphasized across blocks of trials. Consistent with the recent monkey study, we found that the CPP reached a similar threshold before response onset across different SAT regimes. However, the slope of the CCP during decision-making was higher in the speed-emphasized compared to accuracy-emphasized blocks suggesting faster sensory evidence accumulation. Moreover, we found that the CPP peaked right at the response onset in the speed-emphasized block but that it peaked ~50-100ms before the response onset in the accuracy-emphasized block, which suggests that speed pressure reduces non-decision time. Interestingly, we also found that the CCP reached different thresholds when the data in the speed-emphasized block were sorted by short and long reaction times (RTs); however, only the slope of the CCP changed when the data in the accuracy-emphasized block were sorted by RTs. Overall these results challenge the original views on how the SAT impacts decision-making by demonstrating that the SAT regulates multiple - instead of one— components of decision-making processes, and these include the rate of evidence accumulation, non-decision time, and decision boundary.

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Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

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Topic: H.02. Human Cognition and Behavior

Title: Sacrificing evidence for urgency: Tracking stratal activation during mounting urgency during decision-making

Authors: *S. MAASS[1], H. VAN RIJN[2], B. FORSTMANN[3], L. VAN MAANEN[3];
[1]Fac. of Behavioural and Social Sci., Groningen, Netherlands; [2]Exptl. Psychology, Univ. of Groningen, Groningen, Netherlands; [3]Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: As time passes in the process of making a decision, the need to give a response or perform an action increases. This "need-to-act" is defined as a sense of urgency. In the standard models of binary decision-making, noisy evidence is accumulated until a fixed threshold is reached for one of the two choices. Recent work has highlighted the striatum as the main brain region controlling this process (Forstmann, Ratcliff, & Wagenmakers, 2016). In contrast to the concept of fixed thresholds, current evidence suggests that the amount of evidence needed to give a response, might change during the process of making a decision (Boehm, Hawkins, Brown, van Rijn & Wagenmakers, 2015; Cisek, Puskas, & El-Murr, 2009). As time passes and urgency increases, the amount of evidence needed to trigger a decision seems to decrease, leading decision-makers to make a choice based on less evidence. In a first experiment, we tested three methods to induce urgency, which vary in the degree of explicit time pressure: an implicit method of slowing down the presentation of stimuli, a reward-based method in which points can be earned through fast responses and a deadline method in which participants are required to respond before a deadline. Using an expanded judgement paradigm we were able to compute the likelihood ratio of the chosen alternative to quantify the urgency effect. Results of a behavioural experiment show that all methods were able to induce urgency. The implicit method, however, was least affected by confounds such as strategy use. Looking at the neural mechanism behind the urgency effect previous research has shown the basal ganglia to be a likely source (Thura & Cisek, 2016). Our second experiment aims to describe how varying levels of urgency affect the development of activation in the basal ganglia, specifically the striatum, on a single trial level. Accordingly, the implicit urgency inducing method from Experiment 1 was employed in a 7T fMRI experiment. We hypothesize that in trials with a high level of urgency the striatum will be less active than in trials with a low level of urgency. Over time, this difference in activation could result in a lower response inhibition, and therefore responses are made based on less evidence for the chosen option.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIMH DIRP

Title: Striatal dopamine relates to information sampling in the beads task as a measure of impulsivity

Authors: *R. VICARIO FELICIANO, V. D. COSTA, J. B. CZARAPATA, D. EISENBERG, K. BERMAN, B. B. AVERBECK;
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Abstract: Impulsivity refers to the tendency to act without forethought or consideration of action outcomes. Since decisions are most effective after collecting sufficient information to predict rewarding outcomes, information sampling tasks have proven useful as a behavioral metric of impulsivity. The beads task is a probabilistic decision making task used to study information sampling biases. Participants view sequences of bead colors drawn from hidden urns and attempt to infer the majority bead color in each urn. After viewing each bead drawn, participants chose either to seek more evidence about the urn by drawing another bead (draw choices) or to infer the urn contents (urn choices). Functional imaging of the beads task shows that the decision to stop sampling information and guess an urn activates a reward anticipation network that includes the striatum. Further, a subset of Parkinson's disease patients that develop behavioral addictions following dopamine replacement therapy and patients with schizophrenia display reduced information sampling in the beads task. This suggests that dopaminergic signaling contributes to information sampling biases. However, dopaminergic manipulations in healthy controls have not been shown to modulate information sampling behavior in the beads task. To determine if striatal dopamine is related to information sampling we measured presynaptic DA stores and synthesis with [^{18}F]-FDOPA positron emission tomography (PET) in 65 healthy participants and correlated it with information sampling behavior in the beads task. The specific uptake constant, K_i, a measure of dopamine synthesis activity, was estimated for striatal regions of interest (caudate, putamen, and ventral striatum). Striatal dopamine synthesis capacity was inversely correlated with number of beads drawn in caudate and putamen. These results implicate dopamine function in information sampling behavior and therefore implicate increased striatal dopamine in impulsivity.

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Poster

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Support: Strategic Initiatives Grant (The University of Melbourne) to CM and SB

Australian Research Council Discovery Early Career Research Award (#140100350) to SB

Title: The feedback-related negativity encodes an information prediction error in decisions to seek information

Authors: *D. BENNETT, M. BRYDEVALL, C. MURAWSKI, S. BODE;
The Univ. Of Melbourne, Melbourne, Australia

Abstract: In a dynamic world, accurate beliefs about the environment are vital for survival, and individuals ought regularly to seek out new information to update their beliefs. This aspect of behaviour is not well-captured by rational theories of decision making, and the neural mechanisms of information seeking are poorly understood. One recent theory posits that canonical neural reward-processing circuits assign an intrinsic value to information, even when that information lacks instrumental use. We investigated this question by recording EEG from twenty-two participants performing an information-seeking task. In this task, participants could pay a monetary cost to receive advance information about the likelihood of receiving reward in a lottery at the end of each trial. Importantly, acquiring information did not alter reward probability, which was known to participants. Behavioural results showed that participants were willing to incur considerable monetary costs to acquire early but payoff-irrelevant information. Analysis of the event-related potential elicited by informative cues revealed that the feedback-related negativity (FRN), a component strongly linked to reward processing, encoded an information prediction error. Crucially, encoding of the information prediction error was orthogonal to encoding of a reward prediction error, which was also reflected in FRN amplitude. These findings suggest that information may represent a distinct dimension of valuation in decision making under uncertainty, and that information seeking might result from intrinsic valuation of information within neural reward-processing circuits.

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Poster

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Program#/Poster#: 648.20/MMM45

Topic: H.02. Human Cognition and Behavior

Support: NIH

Title: Neurons encode subjective opinion in the human prefrontal cortex.

Authors: *M. JAMALI, Z. MOSES, K. HAROUSH, S. PATEL, Z. WILLIAMS;
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Abstract: Subjective opinion plays a critical role in many social, political and legal arenas, yet they can also dramatically differ between individuals even when observing the same precise situation or scene. How subjective opinions computed by neurons in the human brain is unknown. Here, we investigated neural correlate of subjective opinion in patients undergoing planned neurosurgery by recording from single units in the dorsolateral prefrontal cortex (dlPFC). During recordings, the participants were presented with visual stimuli of seven different real-world scenes, and were asked to render judgments about them. Our data from a total of 134 dlPFC neurons indicated that many neurons in the dorsal lateral prefrontal cortex (dLPFC) closely tracked the subjective opinions of the participants. Whereas certain neurons displayed responses that shifted when transitioning from one opinion to another, other neurons displayed responses that preferentially signaled their 'tipping-point' or equipoise. When grouped together, these neuronal populations provided a remarkably accurate account of an individual's voting profile. They were also predictive of their upcoming selections within less than a second after the scenes were presented. These findings identify a set of basic rules by which subjective opinions are encoded by neurons in the human dlPFC, and may help explain how humans make rapid decisions under conditions in which there are no explicit rules to guide their selections.

Disclosures: M. Jamali: None. **Z. Moses:** None. **K. Haroush:** None. **S. Patel:** None. **Z. Williams:** None.

Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 648.21/MMM46

Topic: H.02. Human Cognition and Behavior

Support: University College London Grand Challenge Award

Wellcome Trust Career Development Fellowship

Title: Deciding to know: information prediction errors and value in the human brain

Authors: *C. J. CHARPENTIER[1], E. S. BROMBERG-MARTIN[2], T. SHAROT[1];
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Abstract: How do we decide what to know and when to remain ignorant? Knowledge about the world is vital for informing our choice of actions. Moreover, knowledge can have an intrinsic value: humans and animals exhibit a strong preference to seek information even when that information cannot be used to guide action. Yet, the mechanisms underlying information seeking in humans are largely unknown. Here, we characterize the neural basis and computational principles of how people decide what to know. We show that the decision to receive information is driven both by the resolution of uncertainty and the likelihood of receiving good news. Participants were more eager to know about outcomes with higher expected value, a strong effect that was also modulated by outcome uncertainty. We identify two sets of Information Prediction Errors (IPE) in the human brain, which represented these two motives. One set of IPEs were independent of expectations of reward and loss (in OFC/vmPFC) and the other (in ventral striatum) were modulated by such outcome expectations. Our results identify the components that give rise to information seeking decisions and their respective encoding in the human brain.

Disclosures: C.J. Charpentier: None. **E.S. Bromberg-Martin:** None. **T. Sharot:** None.

Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

Location: Halls B-H

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Topic: H.02. Human Cognition and Behavior

Support: NUS Grant WBS R-581-000-123-133

NUS Grant WBS R-581-000-133-112

Title: Converting from count to worth: neural mechanisms of the value-to-utility transformation

Authors: *O. A. MULLETTE-GILLMAN, Y. A. KURNIANINGSIH;
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Abstract: Choosing the ‘best’ option is not just selection of the one that is most numerous or physically largest, but requires that the options be converted from such physical measures of objective value (or count) to subjective measures of worth. Here, we demonstrate that the information necessary to perform this value-to-utility transformation is encoded within the the dorsal anterior midcingulate cortex (daMCC), using a highly specific computational model (a

270-point fit, with 15 trials per point). Specifically, for each value, enhanced daMCC activation results in reduced subjective valuation (SV), diminished activation with enhanced SV, and baseline activation with non-modulated SV.
Analyses were performed in a within-study replication design, with initial analyses focused on the gains domain and replication in the losses domain. Participants (N=30) chose between certain and gamble options with varied absolute value and probability (Kurnianingsih et al., 2015). The value-to-utility transformation of each participant was quantified using a standard measure of risk preference (alpha). Participants were on-average risk averse in the gains domain, with a wide range of preferences.
To localize the brain region responsible for the value-to-utility transformation, we identified neural regions that encode a linear value signal whose slope is determined by the direction and degree of subjective valuation each individual is performing. This was accomplished through between-subject covariate analyses, applying individual preference values upon trial-value regressors (two independently tested, both demonstrated to caputre value coding within the ventral medial prefrontal cortex). Whole-brain analyses localized this information to a single cluster of voxels within the daMCC, which replicated in the losses domain. Post-hoc ROI analyses confirmed a zero-centered negative relationship between activation of the daMCC and subjective valuation. Functional connectivity analyses indicate that this region is positively connected with lateral prefrontal cortex and negatively connected with the nucleus accumbens, which we hypothesize allows the value-to-utility transformation to be 'set' by contextual inputs and modulate reward regions.
In conclusion, we show that the daMCC encodes the information necessary to perform the value-to-utility transformation, providing a clear computational function for the daMCC.

Disclosures: O.A. Mullette-Gillman: None. **Y.A. Kurnianingsih:** None.

Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

Location: Halls B-H

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Program#/Poster#: 648.23/MMM48

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01-DA027764

NIH Grant F32-MH107175

Title: Neural pathways underlying explore-exploit tradeoffs in social and nonsocial contexts

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Abstract: Many of our decisions require us to tradeoff using existing information (exploitation) and gathering new information (exploration). Previous work has suggested that exploitative decisions involve the ventromedial prefrontal cortex and the ventral striatum whereas exploratory decisions involve the frontopolar cortex and intraparietal sulcus. While these brain regions help shape explore-exploit tradeoffs, it remains unclear whether such decisions are influenced by social context. We therefore created a social variant of a conventional 2-armed bandit task. On each trial, participants were first presented with a picture of their partner (a confederate or a computer) before choosing one of two bandits where the potential payouts (1-100 points) varied across time according to Gaussian random walks. After selecting a bandit, participants were shown how many points could be won on that trial. Participants were then shown a screen indicating that their partner was playing an unrelated card-guessing game. Next, participants were asked to press a button to reveal whether their partner guessed correctly or incorrectly. If the partner guessed correctly, then the participant received the points; but if the partner guessed incorrectly, the participant did not receive the points. We found that the partner's success on the previous trial increased exploitative decisions, despite being independent from the potential payouts of either bandit. Our preliminary neuroimaging analyses (N = 11) revealed two results. First, dorsal anterior cingulate cortex responses encoded the absolute extent to which the potential points deviated from what the participants expected (i.e., unsigned prediction errors). Second, ventral striatal responses to winning points depended on the type of partner, with the confederate partner evoking more activation to winning points compared to the computer partner. Taken together, our results suggest that both behaviors (i.e., tendency to exploit) and neural pathways (i.e., ventral striatum) underlying explore-exploit tradeoffs can be influenced by social context.

Disclosures: D.V. Smith: None. **S. Wang:** None. **M.R. Delgado:** None.

Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 648.24/MMM49

Topic: H.02. Human Cognition and Behavior

Title: Neural mechanisms underlying self-consistency in social behavior

Authors: *J. FUJIWARA[1], P. N. TOBLER[2], K. I. TSUTSUI[3], M. TAIRA[4], Y. UGAWA[5], S. EIFUKU[1];
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Abstract: To facilitate life in social environments, we often change our decisions and judgments in order to conform to the social group. However, we also value being consistent with ourselves by conforming to our own judgments. Social influence on our behavior has been extensively studied in social psychology. However, the neural mechanisms of self-conformity and self-consistency remain unclear. In this study, we investigated the neural mechanisms underlying group and self-conformity in a sequential facial attractiveness-rating task with functional magnetic resonance imaging. One week after having given ratings of facial attractiveness in the scanner, participants were asked to rate the same faces again. At the second rating, participants were provided with the previous rating of either the "group average" or "yourself". To convey these group or individual ratings to the participants, we first manipulated each participant's previous ratings by splitting them into two conditions; true (same rating as their previous rating) or false (shift above or below their previous rating). We then randomly selected half of the ratings and used them as basis for the group opinion, whereas the other half was used as basis for the individual opinion. In line with previous research, we found that participants adjusted their judgments of facial attractiveness at the second rating to conform to the group opinion. Interestingly, participants also adjusted their judgments to conform to their own opinion stated in the initial ratings. Indeed, comparing group and individual conditions, participants tended to conform at least as much to their own ratings as to those of the normative group. In the brain, false group and false individual reminders triggered distinct activations. Direct comparisons showed stronger temporo-parietal responses for group conformity than self-conformity and stronger medial prefrontal and central orbitofrontal responses for self-conformity than group conformity. These data suggest that social conformity and self-consistency are based on different neural mechanisms.

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Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 648.25/MMM50

Topic: H.02. Human Cognition and Behavior

Title: The social conformity effect uppon liking and sharing behaviors.

Authors: *N. FISCHER[1], B. E.M. DO NASCIMENTO[3], M. FIORANI, Jr.[2];
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Abstract: We are constantly asked to express our opinion. With the popularity of social networks, the expression of preferences can be measurable. However, it is still to be understood what kind of underlying process would be influencing our declared preferences. In this way, our goal is to elucidate what behavioral and neural parameters could be related to two common behaviors expressed by people in social networks: liking and sharing. The experimental design consisted in the visualization of a set of 10 seconds videos, each of them followed by the questions: (1) “How much did you like the video?”; (2) “Would you share this video with someone else?”; and (3) “Have you seen this video before?”. Subsequently, subjects visualized the whole set of videos a second time, but before the questions it was presented a feedback screen shown how that video was supposedly evaluated by others. During the whole experiment, subjects had their behavior and their electroencephalographic (EEG) parameters recorded. Regarding behavioral parameters, regression analysis showed that, at the populational level, thirds’ opinion about videos influenced strongly (p~0) participant’s evaluation. Regarding the ‘like behavior’, the subject’s second responses statistically followed the ‘others’ (feedback) evaluation showing a strong social conformity effect. The feedback also affected significantly the ‘sharing behavior’, so, downgraded videos were less shared while upgraded videos were shared more times. These results show that the conformity with third part’s opinion is an important modulator of “liking” and “sharing” behavior. Regarding EEG data, it is still under analysis.

Disclosures: **N. Fischer:** None. **B. E.M. do Nascimento:** None. **M. Fiorani:** None.

Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

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Program#/Poster#: 648.26/MMM51

Topic: H.02. Human Cognition and Behavior

Support: 2015BCA0120

1.150122.01

Title: Neural correlates of conforming behavior toward a group sharing the same preference on movies

Authors: *S. LEE, D. LIM, E. SEOMOON, D. KANG, S.-P. KIM;
UNIST, Ulsan, Korea, Republic of

Abstract: Humans estimate the value of objects and make judgment based on it, which is often affected by both internal and external factors. Other's opinion is one of the external factors that can change one's decisions. Many studies have shown that individuals conform to the opinion of a reference group such as public, experts or peers, driven by a desire to belong to the reference group. In line with the previous studies, we aimed to investigate how individuals conform to a reference group sharing the same preference for movies that they frequently consume in daily life. Also, we aimed to examine neural signals modulated by such conforming behavior. In the beginning, participants assessed a list of movies they watched before and were informed that they belonged to one of the four groups based on their assessments. Next, participants performed a series of trials in which participants were shown a movie poster and asked to evaluate how much they anticipated to see the movie, then were exposed to the assessment of the assigned reference group, and finally reevaluated the movie while seeing the same poster again. EEG signals were collected while participants were informed of the assessment of the reference group. There were two behavioral patterns when participants encountered a conflict with the reference group: changing evaluation toward the reference group or keeping their initial ratings. A negative deflection in the frontal area (Fz) peaking at 300 ms, which is known as feedback related negativity (FRN), was increased when participants conformed to the reference group. It reflects a prediction error signal in the reinforcement learning model, induced by a difference between expected outcome (i.e. initial rating) and obtained outcome (i.e. assessment of the reference group). It was shown that participants' adjustment was bounded to the assessment of the reference group, indicating a possibility of the anchoring effect occurring during conformity to the reference group. Our findings suggests that a reference group sharing the same preference can lead to conforming behavior.

Disclosures: S. Lee: None. **D. Lim:** None. **E. Seomoon:** None. **D. Kang:** None. **S. Kim:** None.

Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

Location: Halls B-H

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Program#/Poster#: 648.27/MMM52

Topic: H.02. Human Cognition and Behavior

Title: Temporal dynamics of decision-making for oneself versus others

Authors: *A. M. HARRIS[1], C. HUTCHERSON[2];
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Abstract: Although research on decision-making has focused largely on choosing for oneself, in our daily lives we must often select options for others: for example, preparing a meal for a child or buying lunch for a friend. In these cases, we must take into account another's preferences, even though they may differ markedly from our own. Yet, it is unclear when such attributions of others' preferences emerge in the time course of choice. Here we measured participants' brain activity with event-related potentials (ERP) while they made food choices for themselves and two partners, one with similar tastes and one with markedly different (i.e., "health-conscious") preferences. Behaviorally, participants changed their weighting of taste and health attributes depending on the intended recipient, relying most heavily on taste for themselves and on health for the health-conscious partner. As seen previously, neural value signals were visible from 500 to 650 ms after stimulus onset, and were localized to ventromedial prefrontal cortex (vmPFC), a brain region implicated in valuation. Neural activity differentiating decisions for oneself versus others emerged even earlier, from approximately 300-500 ms post-stimulus, localized to social cognition regions including posterior superior temporal cortex (pSTC). However, when individual taste and health ratings were included as covariates, early activity for oneself versus other was largely explained by the weighting on health information. In contrast, neural responses to taste emerged in the later time window associated with value, and showed no interaction with the intended recipient. Consistent with this idea, value-related brain activity showed a significant interaction of the weighting of taste and health information by the intended recipient. Whereas taste contributed most strongly to ERPs for oneself, health information was weighted increasingly with increasing partner distance (Self < Similar < Different). Together, these results provide novel insights into the time course of decision-making when others' preferences are taken into account.

Disclosures: A.M. Harris: None. **C. Hutcherson:** None.

Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

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Topic: H.02. Human Cognition and Behavior

Support: NIH R01 MH091068

NIH U54 HD079125

Title: Altered striatal activity to wins and losses of rewards in ADHD

Authors: *C. FASSBENDER[1], P. MUKHERJEE[1], C. A. CALUB[1], J. F. DIXON[1], S. HINSHAW[2], A. E. GUYER[3], W. VAN DEN BOS[4], S. MCCLURE[5], J. B. SCHWEITZER[1]; [1]UC Davis Sch. of Medicine, MIND Inst., Sacramento, CA; [2]Dept. of Psychology, Univ. of California Berkeley, Berkeley, CA; [3]Human Develop. and Family Studies Unit, Dept. of Human Ecology, UC Davis Ctr. for Mind and Brain, Davis, CA; [4]Max Planck Inst. for Human Develop., Berlin, Germany; [5]Psychology, Arizona State Univ., Tempe, AZ

Abstract: ADHD impairments are associated with altered motivation, perhaps attributable to altered response to rewards in this group. However, many tasks used to evaluate reward processing also involve cognitive control processes, also implicated in ADHD impairment. We employed fMRI and a Numeric Guessing Game reward paradigm to examine neural responses to wins and losses in youth with ADHD compared to typically developing peers (TD). Participants consisted of 20 ADHD (mean age 14.83 yrs) Combined subtype (significant symptoms of inattention and hyperactivity/impulsivity) and 20 (mean age 14.82 yrs) age and sex-matched TD peers between the ages of 12 and 18. A Numeric Guessing Game presented participants with a single digit number between 0 and 9 and they were required to guess, by button press, if a "hidden" number was greater than or less than the presented number. The response period was followed by a variable "anticipation" period (2, 4 or 6 sec; avg. 4.13 sec). Participants were then provided feedback; a smiling face represented a correct guess and a $1 reward and a frowning face represented an incorrect guess and a loss of 50c. An entire trial was followed by a jittered ITI (4,6 or 8 sec; avg. 6.13 sec). Trials were presented in 4 blocks of 15 trials each for a total of 60 trials.
ADHD and TD groups did not differ on age, Tanners pubertal ratings or Full scale IQ. The ADHD group were significantly elevated on Conners' parent ratings of both inattention and hyperactivity/impulsivity ($p<0.0001$) compared with the TD group. AFNI preprocessed and analyzed the imaging data. Following the GLM, we performed a 2 (Condition; Win v Loss) x 2 (Group; TD v ADHD) ANOVA, which examined the Group x Condition interaction, as well as the within group Win v Loss maps. The TD Win v Loss map revealed a number of brain regions including multiple regions in the striatum (caudate, putamen, lentiform nucleus), the insula, inferior frontal gyrus (IFG) and anterior cingulate cortex (ACC). The ADHD Win v Loss map revealed two regions one in the IFG and one in the ACC. The Group x Condition Interaction map revealed two regions in the striatum and one in the ACC. We extracted percent signal change values from the striatal ROIs, due to their prior importance in the reward literature and in ADHD. The TD group displayed greater activity in all striatal regions to wins over losses whereas the ADHD group displayed equivalent activity in the striatum to wins and losses (Left Caudate and Right lentiform nucleus).
These results suggest a blunted response in the striatum to wins and losses in ADHD. This may explain why individuals with ADHD respond differently to rewards compared with their typically developing peers.

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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; Shire Pharmaceuticals.

Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

Location: Halls B-H

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Topic: H.02. Human Cognition and Behavior

Support: Pennsylvania State University Childhood Obesity Training Program funded by USDA National Institute for Food and Agriculture Grant #2011-67001- 30117 Program A2121

SLEIC Dissertation Award, Pennsylvania State University

Title: Differences in brain response to anticipation for food and money rewards predicts children's intake of savory foods served at a highly palatable buffet meal.

Authors: *S. ADISE[1], A. M. CAPRIO[2], N. J. ROBERTS[3], C. N. WHITE[4], C. F. GEIER[3], K. L. KELLER[1];
[1]Nutritional Sci., [2]Biol., [3]Human Develop. and Family Studies, The Pennsylvania State Univ., University Park, PA; [4]Psychology, Syracuse Univ., Syracuse, NY

Abstract: Basic decision-making is integral for food choice. fMRI studies show that brain regions implicated in decision-making (e.g., reward and inhibitory control [IC]) respond to food cues, and increased response is associated with obesity. Thus, brain response in regions associated with reward and IC may underlie food-based decisions but how this relates to actual food intake is unknown. Previously, we found that selection of savory-fat food items (e.g., pizza, chips, etc.) over sweet and sweet-fat foods at a highly palatable buffet positively correlated with weight status. However, the neural mechanisms underlying these food-based decisions have not been determined. We hypothesized that increased intake of savory-fats and child body mass index (BMI) *z*-score would correlate with BOLD signal in regions associated with reward processing (e.g. medial prefrontal cortex [mPFC], ventral medial prefrontal cortex [vmPFC]) and IC (e.g. dorsolateral prefrontal cortex [dlPFC]) when anticipating food > money (F_a>M_a) rewards. We measured fMRI BOLD response to anticipation of food and money rewards (via a modified card-guessing task), BMI *z*-score, and *ad libitum* intake at a highly palatable buffet of savory (e.g., pizza, chips), sweet (e.g., candy), and sweet-fat (e.g., cookies) foods in 7-11-year-old children (n=26; 42% obese/overweight). Multiple linear regressions were used to predict intake with independent predictors of BMI *z*-score and BOLD activation in the regions associated with reward and IC to F_a>M_a. Results showed that intake of savory-fats was positively

predicted by BMI *z*-score (p=.046) and $F_a>M_a$ BOLD signal in the mPFC (p=.019), which explained 33% of the total variance in intake (*p*=.009). vmPFC BOLD signal for $F_a>M_a$ (p=.05) and BMI *z*-score (p=.046) positively predicted intake, explaining 28% of variance (p=.02). Savory-fat intake was also positively predicted by BMI *z*-score (p<.001) and BOLD signal to $F_a>M_a$ in the left dlPFC (p=.001) (explaining 48% of total model's variance [p=.001]) and right dlPFC (p=.02) (predicting 50% of the total model's variance in intake of savory-fats [p<.001]). There were no relationships between the aforementioned regions and intake of the total meal, sweet, or sweet-fat foods. Our results suggest that anticipation of food relative to money rewards in brain regions implicated in reward and IC predicts intake of savory-fat foods at a buffet meal. Therefore, brain regions involved in decision-making may play a crucial role in overeating, particularly for savory items. Further investigation into the mechanisms underlying brain responses to rewards are driving increased food intake is needed.

Disclosures: S. Adise: None. **A.M. Caprio:** None. **N.J. Roberts:** None. **C.N. White:** None. **C.F. Geier:** None. **K.L. Keller:** None.

Poster

648. Decision Making: Information, Impulsivity, and Social Rewards

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Topic: H.02. Human Cognition and Behavior

Support: NIMH Conte Center Grant on the neurobiology of social decision-making

JSPS fellowship for research abroad

Title: Elucidating the underlying components of food valuation in the human orbitofrontal cortex

Authors: *S. SUZUKI[1,2], L. CROSS[3], J. P. O'DOHERTY[2,3];
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Abstract: The valuation of food is a fundamental component of the decision-making process that all humans complete on a daily basis. Consumption of high nutrient food increases the likelihood of survival, while consumption of low quality food is deleterious for health. A dysfunctional food valuation process may play a large role in the development of obesity, diabetes, and eating disorders. The orbitofrontal cortex (OFC) is thought to be a canonical brain region for food valuation. There is accumulating evidence that the OFC and adjacent medial frontal cortex represents information about value for various classes of goods including food.

Also, the OFC receives direct projections from primary sensory areas involved in representation sensory features of food such as gustatory and olfactory cortex. Yet, little is known about how value of a food is computed in the OFC. Specifically, it remains elusive how the OFC computes a food value through integrating information about multiple nutrient factors.
To address this issue, we scanned 24 human participants by fMRI while they reported their "willingness to pay" (i.e., value) for 56 food items (Task 1), followed by a behavioral task in which they rated subjective nutrient factors of the same set of the items (Task 2). Importantly, during Task 1, the participants were not aware that they would be subsequently required to rate nutrient factors of the items. Behaviorally, we found that subjective ratings about the four nutrient factors, fat, carbohydrate, vitamin and protein, predicted subjective value of the food items. Furthermore, multivariate analyses (MVPA) on the fMRI data revealed that information about food value can be decoded above chance-level from patterns of neural activity in both medial and lateral parts of the OFC. On the other hand, information about the nutrient factors can be significantly decoded from neural activity in the lateral OFC, but not in the medial OFC. These results may suggest that food value is computed in lateral OFC by integrating information about multiple nutrient factors and sent to medial OFC to guide behavior.

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Poster

649. Human Cognition: Aging II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 649.01/MMM56

Topic: H.02. Human Cognition and Behavior

Support: NIAAA Grant: R01AA021187

Title: Effects of sex and education on trajectories of cognitive change with age in older adults

Authors: *L. K. MCEVOY[1], E. T. REAS[2], J. BERGSTROM[3], D. KRITZ-SILVERSTEIN[3], E. BARRETT-CONNOR[3], G. A. LAUGHLIN[3];
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Abstract: Many cognitive abilities decline with advancing age. However, the magnitude and timing of cognitive changes, and their moderators, are not fully known. In this study we aimed to characterize sex-specific trajectories of change in cognitive performance over a 27 year follow-up period in older community-dwelling adults, and to assess the influence of education on age-related cognitive decline. Between 1988 and 2016, 2225 community-dwelling participants of the

Rancho Bernardo Study (mean age 71 years, range 31-99 years at initial cognitive assessment) completed up to seven cognitive assessments at approximately 4 year intervals. Participants completed tests of global cognition, executive function, category fluency, and verbal episodic memory. Linear mixed effects regression models were used to define sex-specific trajectories of cognitive change with age, adjusting for education and retest effects. Analyses were conducted on the full cohort, and after excluding individuals with evidence of cognitive impairment at the first or last cognitive assessment. Cognitive impairment was defined as an MMSE score greater than two standard deviations below the sex-, age-, and education-adjusted mean, based on normative data from the National Alzheimer's Coordinating Center (Shirk et al. *Alzheimer's Research & Therapy,* 2011). Significant decline was observed on all tests for both men and women, with acceleration in decline beginning around age 65 and increasing after age 80. Higher education was associated with slower decline in executive and global function for both sexes, but was not associated with rates of decline in category fluency or episodic memory. Although women performed better than men on the episodic memory test, rates of decline did not differ between men and women. Men were more likely than women to show cognitive impairment (32% vs 17%). When these 517 participants were excluded, age-related decline on all tests remained significant, and women showed steeper decline in executive function than men. This study shows that significant decline with age occurs across multiple cognitive domains in men and women, with men and women showing similar trajectories of decline in most domains. Higher education protects against age-related decline in some cognitive domains, but not in others. In future studies we will take advantage of the wealth of information available in this extremely well-characterized longitudinal cohort study to examine associations of a wide range of health and lifestyle variables with trajectories of cognitive change with age, and will investigate whether these associations differ by sex.

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Poster

649. Human Cognition: Aging II

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Topic: H.02. Human Cognition and Behavior

Support: ŇİÅÅÅ Ģřåňţ: Ř01ÅÅ021187

Title: Effects of apoe status on age-related cognitive decline in community-dwelling older adults: modulation by sex, education and lifestyle

Authors: *E. T. REAS[1], G. A. LAUGHLIN[2], J. BERGSTROM[2], D. KRITZ-SILVERSTEIN[2], E. BARRETT-CONNOR[2], L. K. MCEVOY[1];
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Abstract: The Apolipoprotein E (ApoE) gene is one of the strongest genetic risk factors for sporadic Alzheimer's disease, with elevated disease risk for carriers of the E4 allele. However, there is conflicting evidence about whether E4 accelerates cognitive decline in normal aging. We assessed the association between ApoE genotype and age-related cognitive decline in community-dwelling adults from the Rancho Bernardo Study, and assessed whether E4 effects were modulated by sex, education and lifestyle. Tests of global cognitive function, executive function, verbal fluency and episodic memory were administered to 1393 adults (1087 E4-; 306 E4+) aged 44-99 years at their initial cognitive assessment. Participants were evaluated up to seven times over a maximum follow-up period of 27 years between 1988 and 2016. Linear mixed effects models examined age-related trajectories of cognitive change, assessing the influence of ApoE genotype, sex, education, physical activity, alcohol consumption and smoking. There were no main effects of ApoE on cognitive function, but E4 carriers showed greater age-related decline than non-carriers on tests of executive function and verbal fluency for both men and women. Presence of an E4 allele in men, but not in women, was associated with greater age-related decline in global cognitive function. Verbal fluency decline was accelerated for those without a college education, but only for E4 carriers. E4 interacted with alcohol consumption on age-related decline in global cognitive function and memory, but did not interact with smoking or physical activity. These findings indicate that the E4 allele may increase risk for age-related decline. This risk differs between men and women, and may be modulated by education and alcohol consumption.

Disclosures: E.T. Reas: None. **G.A. Laughlin:** None. **J. Bergstrom:** None. **D. Kritz-Silverstein:** None. **E. Barrett-Connor:** None. **L.K. McEvoy:** None.

Poster

649. Human Cognition: Aging II

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Program#/Poster#: 649.03/MMM58

Topic: H.02. Human Cognition and Behavior

Title: Alzheimer's disease risk factor score is associated with cognitive performance and brain volume

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Abstract: Background: Large epidemiological studies suggest there are 7 main modifiable lifestyle factors that increase the risk of developing Alzheimer's disease (AD): obesity, low education, physical inactivity, diabetes, hypertension, and depression. However, it is unknown whether the presence of these risk factors in cognitively normal individuals is associated with early changes in cognition or brain structure. The objective of this study was to create a composite risk factor score (RFS) and correlate it with performance on cognitive tests of attention, executive function, and memory in addition to brain structure across the lifespan.
Methods: Participant data (N=379, Ages 22-85) were obtained from a cross-sectional study performed by the Nathan Kline Institute. The RFS was calculated as a weighted sum of these risk factors, with weights based on previously reported relative risk values (Barnes & Yaffe, 2011); a higher RFS suggested greater AD risk. RFS was correlated to performance on the following cognitive tests: Forward and backward digit span, DKEFS Trails A and B, and RAVLT delay and immediate recall. T1 MRI images were processed using voxel based morphometry. A linear regression analysis was performed to correlate RFS with grey matter (GM) volume, controlling for age. The RFS was also split at the median; a 2 sample t-test was performed to evaluate group differences (lower vs. higher RFS) in voxel-wise brain volume across the lifespan. A subgroup analysis was performed for 3 age groups: early-life (EL) (22-39), mid-life (ML) (40-64), and late-life (LL) (65-85).
Results: The RFS distribution showed a slight leftward skew towards protection against AD across all participants. Trails A and B tests showed a positive association with RFS across all participants and in the LL subgroup analysis. A negative RFS association was seen with the forward digit span in the ML group. RFS was not significantly associated with RAVLT. Linear regression analysis showed negative associations between GM volume and RFS in the precuneus (LL), thalamus (ML), and basal ganglia (EL) ($p<.001$). The RFS median split analysis showed lower GM volume in the amygdala, medial-prefrontal area, and inferior temporal gyrus for those with higher RFS (LL only).
Conclusions: Our study suggests that these modifiable AD risk factors are associated with cognitive function and brain volume across the lifespan, even in cognitively normal older adults. Lower GM volume observed in the precuneus, medial-prefrontal cortex, and inferior temporal cortex could indicate increased vulnerability to developing AD as these are known regions of beta-amyloid and tau deposition.

Disclosures: A. Sakhare: None. **W. Mack:** None. **J. Pa:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: German Research Foundation WE 4269/3-1

Max Planck International Research Network on Aging

Max Planck Society: Minerva Research Group

Title: A role for sleep-dependent consolidation in age-related episodic memory decline?

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Abstract: Sleep after learning benefits memory. With advancing age, both sleep and memory performance undergo drastic changes. However, whether the observed age-related alterations result from qualitatively different mechanisms or simply reflect quantitative changes in the reliance on a functionally comparable neurocognitive machinery remains obscure. In the current study, tracing learning histories across multiple learning-test cycles allowed for close monitoring of the strength of individual memories. Hence, we were able to disentangle the effects of overnight forgetting from active enhancement of initially labile memory traces. Particularly, we asked whether aging differentially affects forgetting and active enhancement. To address this question, we analyzed data from 29 younger (YA, 16 female, M = 23.55 years, SD = 2.58) and 36 older adults (OA, 16 female, M = 68.77 years, SD = 3.15) who completed an associative memory paradigm, comprising of a learning session with multiple learning-test cycles as well as a delayed cued-recall task on the following day. During the nights before and after learning sleep was monitored by ambulatory polysomnography (PSG). We focused on the distribution of sleep stages as well as the occurrence of sleep spindles and slow wave activity (SWA) during non-rapid eye movement (NREM) sleep. While overnight memory enhancement was comparable across age-groups, forgetting was more pronounced in OA. Despite altered sleep architecture in OA, there were no age differences in the association between sleep physiology and memory performance. Crucially, SWA and the occurrence of sleep spindles were related to overnight forgetting across the whole sample. Reduced SWA and spindle density in the elderly were paralleled by decreased overnight memory retention, most prominently expressed for pairs of poor memory strength. Contrary, no reliable association between memory enhancement and

measures of sleep physiology could be detected. The present findings suggest two distinct consolidation mechanisms that differ with regard to their relation with sleep and aging. Our results underpin the idea that functionally similar mechanisms underlie the consolidation of episodic memories in both younger and older adults. Specifically, slow oscillations and sleep spindles during NREM sleep counteract overnight forgetting. The reduction of SWA and sleep spindles in OA likely impedes the reactivation of memory traces during sleep and their redistribution to the brain's long-term store in the neocortex. Hence, while overnight memory retention in the elderly is impaired, memory enhancement remains spared from aging.

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Poster

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T32 AG027668

DoD NDSEG

Title: Lateral entorhinal cortex hypoactivation in amnestic mild cognitive impairment

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Abstract: Increased hippocampal activation in the context of impaired memory function is considered a characteristic feature of the amnestic mild cognitive impairment (aMCI) phase of Alzheimer's disease. However, the entorhinal cortex, which serves as the primary relay for both input and output to and from the hippocampus, is the site of the earliest pathological changes including neuronal, synaptic and volume loss. Specifically, the lateral entorhinal cortex is also the site of significant accumulation of tau neurofibrillary tangles early in the disease progression. To assess whether functional changes can be observed in the lateral and medial entorhinal cortex in patients with aMCI, 37 healthy older adults and 42 patients with aMCI completed a forced

choice memory task designed to tax medial temporal lobe memory function while completing a high-resolution functional magnetic resonance imaging (fMRI) scan. Consistent with their diagnosis and previous studies, patients with aMCI showed a significant memory impairment compared to age-matched healthy control subjects as well as increased hippocampal activation. In this context, patients with aMCI also showed significantly decreased activation in both the left and right entorhinal cortex. The observed hypoactivation was localized to the lateral entorhinal cortex and was not observed in the medial entorhinal cortex. Decreased activation in the left lateral entorhinal cortex was correlated with poorer performance on the memory task in patients with aMCI. Finally, the volume of both the left and right lateral entorhinal cortex was significantly reduced in patients with aMCI compared to healthy control subjects while no volume differences between the groups were observed in the medial entorhinal cortex. These results show that, consistent with the locus of early disease related pathology, decreased activation and reduced volume in the lateral entorhinal cortex is observed in patients with aMCI and is associated with impaired memory function in these patients.

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Poster

649. Human Cognition: Aging II

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Topic: H.02. Human Cognition and Behavior

Support: NIA AG025526

NIA AG19610

State of Arizona DHS

Title: Differential effects of hypertension status and white matter hyperintensity volume on white matter integrity in older adults

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Abstract: Healthy aging has been associated with a greater prevalence of vascular risk factors, such as hypertension and increased cerebral white matter hyperintensity (WMH) burden. We sought to investigate whether hypertension status and WMH volume would differentially affect regional white matter integrity in a sample of 48 neurologically healthy community dwelling older adults, 70-89 years of age, with treated hypertension (N = 25) and without hypertension diagnosis or treatment (N = 23). Groups were matched in age, gender, and education. MRI scans were acquired at 3T, including a volumetric T1, T2 FLAIR, and diffusion-weighted imaging. The volumes of WMHs were computed with a multispectral, automated lesion segmentation method to produce probability maps using Statistical Parametric Mapping (SPM12) and the lesion segmentation toolbox (LST; Schmidt et al., 2012). Diffusion-weighted MR images were processed using TRACULA with Freesurfer, which performs automated probabilistic tractography and generates estimates of fractional anisotropy (FA) and mean diffusivity (MD) for major white-matter pathways (Yendiki et al., 2011). The results indicated that hypertension status, but not WMH volume, was significantly related to FA in the forceps minor ($p < 0.001$) and to MD in the forceps major and minor ($p's < 0.001$). Additionally, WMH volume, but not hypertension status, was significantly associated with FA in the left superior longitudinal fasciculus parietal bundle, right cingulum-angular bundle, and right uncinate fasciculus ($0.021 \leq p \leq 0.049$). WMH volume was significantly associated with MD in the left inferior longitudinal fasciculus ($p < 0.01$). There were no interaction effects of hypertension status and WMH volume on the white matter diffusion measures. Together, these findings suggest that vascular risk factors common in healthy aging may have a differential regional impact on white matter integrity in older adults. Further research is needed to evaluate the longitudinal effect of these vascular factors on metrics of white matter integrity and on cognition in the context of healthy cognitive aging.

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Poster

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Topic: H.02. Human Cognition and Behavior

Title: Event related potentials N500 is decreased in elder people with excess of absolute theta power in a counting Stroop task

Authors: *S. SÁNCHEZ MOGUEL[1], G. ALATORRE CRUZ[2], S. CASTRO CHAVIRA[1], M. GONZÁLEZ LÓPEZ[1], T. FERNÁNDEZ HARMONY[1];
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Abstract: During healthy aging, inhibitory processing is affected at sensorial, perceptual and cognitive levels. Decay in inhibitory efficiency contributes to performance decline in elderly adults observed in tasks requiring selective attention, working memory or sustained attention. Moreover, patients with mild cognitive impairment (MCI) frequently show an even more diminished control of the inhibitory processing. The Stroop task during Event Related Potentials (ERP) has been used to study age-related decline in the efficiency of inhibitory processes. The magnitude of the Stroop effect increases in late adulthood. Studies using ERP have found that the N500 amplitude is attenuated in healthy elderly adults as compared to young adults. However, inhibitory processing in older adults at the preclinical stages of cognitive decline has not yet been assessed. An excess of theta activity, as evaluated by normative Quantitative Electroencephalography (QEEG), is an excellent predictor of cognitive deterioration during aging; which enables the assessment of inhibitory processing years before the appearance of cognitive decline. The aim of this study was to evaluate inhibitory processing in older adults with excess theta Absolute Power as compared to older adults with normal EEG activity. ERP were recorded while subjects performed a counting Stroop task. We found that N500 amplitude was significantly reduced in older adults with excess theta. However, there were not differences in the number of correct answers or in response latency. Previous studies showed that reduced N500 amplitude over the midline frontocentral region during interference trials co-occurs with age-related inhibitory-processing decline. Thus, our results suggest that subjects with excess of theta EEG activity, exhibit electrophysiologically differences in inhibitory attentional processing, as evidenced by ERP. Though these results were not confirmed by behavioral assessment, the electrophysiological differences suggest hidden deterioration of inhibitory processing in subjects at preclinical stages of cognitive decline, which is consistent with previous findings of inhibitory-processing deterioration in MCI and dementia patients. Acknowledgements: Héctor Belmont, Lourdes Lara, Leonor Casanova, PAPIIT (IN225414) and CONACYT (registry no. 245313).

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: CONACYT 238826

UNAM DGAPA PAPIIT IG300115

Title: Neural connectivity during spatial source memory retrieval in young and old adults

Authors: *S. CANSINO[1], P. TREJO-MORALES[1], C. ESTRADA-MANILLA[1], E. H. PASAYE-ALCARAZ[2], E. AGUILAR-CASTAÑEDA[3], P. SALGADO-LUJAMBIO[3], A. L. SOSA-ORTIZ[3];
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Abstract: The memory of our personal experiences is referred to as episodic memory. Personal memories include the event and contextual details, as the moment, place and the emotional state of the individual. The ability to retrieve contextual details declines gradually with advancing age, whereas the ability to remember the event without contextual information is almost unaffected in old age. The former is termed as recollection and the latter as familiarity. Several regions exhibited greater activity in young adults than in old adults during the recollection of episodic information. However, less is known about how the interaction among these regions accounts for the decline of recollection in old age. Twenty-two young adults and 22 old adults participated in the study. Functional magnetic resonance imaging (fMRI) data were recorded while participants performed a spatial source memory task. FMRI data were analyzed using dynamic causal modeling (DCM) to examine neural connectivity during successful and unsuccessful spatial source memory retrieval. DCM analyses were conducted in brain regions that showed significant activity differences between age groups in the contrast between correct versus incorrect source judgments. Only brain activity in regions from the left hemisphere was analyzed. Effective connectivity between the hippocampus and the middle frontal gyrus was reduced in old adults relative to young adults during source retrieval. The results suggest that age effects on recollection cannot be attributed only to local under-recruitment activity but also to a less functional network.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIA Grant AG19610

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State of Arizona DHS

Title: Differential regional alterations of white matter integrity in healthy cognitive aging

Authors: *P. K. BHARADWAJ[1,2,7], L. A. NGUYEN[1,2,7], K. A. HAWS[1,2,7], G. A. HISHAW[3], T. P. TROUARD[4], G. E. ALEXANDER[1,2,5,6,7];
[1]Psychology, [2]Evelyn F. McKnight Brain Inst., [3]Dept. of Neurol., [4]Biomed. Engin., [5]Physiological Sci. Grad. Interdisciplinary Program, [6]Neurosci. Grad. Interdisciplinary Program, Univ. of Arizona, Tucson, AZ; [7]Arizona Alzheimer's Consortium, Phoenix, AZ

Abstract: White matter (WM) microstructural integrity assessed with diffusion weighted imaging is diminished in the context of healthy aging. Such age effects have often shown an anterior-posterior gradient and may be influenced by vascular risk factors such as hypertension. The aim of this study was to investigate regional differences in WM integrity measured by fractional anisotropy (FA) and mean diffusivity (MD) in a healthy sample of community dwelling older adults, ranging in age from 50 to 89 years. The study sample comprised 74 healthy older adults divided into four age groups (50-59, 60-69, 70-79 and 80-89 years). Only neurologically healthy participants without a clinical diagnosis of diabetes or hypertension were included. T1-weighted and diffusion weighted images were acquired at 3T and processed using FreeSurfer (Fischl et al., 2004a) and TRACULA (Yendiki et al., 2011), to create individual ROIs, perform probabilistic tractography, and compute regional values of FA and MD for 18 major WM tracts. The diffusion metrics were tested using ANCOVA with age as the between group factor and total cortical WM volume as a covariate. The results showed that FA decreased with age bilaterally in the anterior thalamic radiation (ATR, $0.011 \leq p \leq 0.021$), and the left temporal and parietal branches of the superior longitudinal fasciculus (SLFT, $p = 0.005$; SLFP, $p = 0.05$). MD demonstrated age-related increases bilaterally in the ATR ($0.003 \leq p \leq 0.014$), cingulum angular bundles ($0.002 \leq p \leq 0.042$), inferior longitudinal fasciculus ($0.001 \leq p \leq 0.006$), uncinate fasciculus ($0.0003 \leq p \leq 0.001$), and the right SLFT ($p = 0.043$). Pairwise comparisons of FA and MD in these tracts showed varying patterns of age group differences that differed between the two diffusion metrics. These results demonstrate that measures of WM microstructure assessed by FA and MD differ by age group, for select tracts, as well as by which

specific age groups are most affected. Together these findings suggest that measures of diffusivity (MD) and microstructural organization (FA) may show differences in their ability to detect age-related WM integrity changes among healthy older adults.

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Poster

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Support: NIH Grant MH080833

Title: Considering structural complexity as a measure of age-related differences in brain morphology

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Abstract: INTRODUCTION: Different measures of cortical morphology have been shown to index distinct aspects of inter-individual differences (e.g., volume, thickness, surface area, gyrification). Here we consider the additional measure of structural complexity, as quantified by fractal dimensionality. Using several open-access MRI datasets, providing a combined sample of over 2000 adults across the adult lifespan, we examined the relationship between fractal dimensionality and other extant measures of brain morphology. We additionally examine the relationship between each measure and age-related differences in brain morphology. In a separate set of analyses we further examine the test-retest reliability of each measure using datasets where participants were scanned multiple times within a short period, either on the same scanner or at a different site (i.e., travelling subjects). METHODS: Common measures of cortical and subcortical structure were measured using FreeSurfer. We additionally calculated the fractal dimensionality of these cortical and subcortical structures using the calcFD toolbox, which we recently developed and freely distribute (http://cmadan.github.io/calcFD/). RESULTS: With respect to cortical structure, we found that fractal dimensionality captured more age-related differences in morphology than either cortical thickness or gyrification (see Madan & Kensinger, 2016, NeuroImage). Additionally, all measures have high test-retest reliability. Age-related differences in subcortical structures was explained better by fractal dimensionality than differences in volume (ICV-corrected). Complexity of ventricular structures was not improved

relative to volume differences, suggesting that the measure may only be indicative of more meaningful age-related differences in neuroanatomical structure. DISCUSSION: When examining the relationship between brain morphology and inter-individual differences, it is important to consider the most appropriate measure. For instance, it has been established that age-related differences are reflected most in cortical thickness, rather than surface area or volume. Here we found that fractal dimensionality, which incorporates shape-related properties, to be a more sensitive measure of age-related differences in cortical and subcortical structures. However, it is important to consider that more age-related variability explained may not be necessarily be desired, as this leaves less variance available to be related to other factors, e.g., performance on cognitive measures, so extant measures may still be a preferable depending on the research question.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIA AG025526

NIA AG19610

Title: Relation of physical sport activity to cognitive performance in older adults

Authors: *M. FRANCHETTI[1,6,7], P. K. BHARADWAJ[1,6,7], L. A. NGUYEN[1,6,7], K. A. HAWS[1,6,7], M. C. FITZHUGH[1,6,7], G. A. HISHAW[2], D. A. RAICHLEN[3], G. E. ALEXANDER[1,6,7,4,5];
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Abstract: It is well-known that aging is associated with differences in cognitive performance among community-dwelling older adults, with executive function, memory, and processing speed often preferentially affected. Physical activity is one factor that may have an important role in influencing observed individual differences in cognitive aging. We sought to determine whether higher levels of self-reported physical sport activity is associated with better

performance on those measures of cognitive function often affected by healthy aging. In a sample of community-dwelling, neurologically healthy older adults, self-report ratings of physical sport activity were obtained from 202 participants ages 50-89 years (mean ± SD = 70.24 ± 10.5 years). Subjects who reported a high level of sport activity (n=38) were compared to those who reported a low level of sport activity (n=164). The groups did not differ in age and years of education. Regression was used to test the effects of age, physical activity, and their interaction. Results revealed significant main effects for age across all domains (p's < 0.0001), but not for physical activity (p's > 0.282). Significant age by physical activity interactions were observed for measures of executive function (WAIS-IV Matrix Reasoning, Stroop Word-Color Interference, and Trails-B; $0.004 < p < 0.032$), memory (Rey Complex Figure Test-Delay Recall; $p = 0.039$), processing speed (Finger Tapping (non-dominant hand); $p = 0.02$), visuospatial function (WAIS-IV Block Design; $p = 0.006$) and language (WAIS-Similarities; $p = 0.017$). These effects remained significant after controlling for hypertension status. The older adult group (72-89 years) with high physical activity performed better than those with low physical activity. In addition, the older adult group with high physical activity performed better than, or comparable to, the younger adult group (50-71 years) with high physical activity. These findings suggest that engagement in high levels of sport activity may be an important element for maintaining cognitive abilities in old age.

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Poster

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Program#/Poster#: 649.12/NNN5

Topic: H.02. Human Cognition and Behavior

Title: Adaptive computerized cognitive training for healthy older adults

Authors: *S. RABIPOUR[1], M. PETRUCELLI[2], M. DE O. G. GERMANO[3], A. POPESCU, K1N 9A8[2], P. DAVIDSON[4];
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Abstract: Computerized cognitive training is becoming increasingly popular and practical, holding promise for both patients and healthy individuals. Nevertheless, basic questions remain about the real-world benefit of such programs and the degree to which participant expectations might influence training effects. Here we examined: i) the feasibility of a 5-week computerized

cognitive training intervention in healthy older adults; and ii) the potential effects of participant expectations on performance during training. Participants were 91 healthy older adults 59-91 years of age (M = 68.88, SD = 6.32; 69 women) randomly assigned to complete either the intervention or an active control program. The intervention, a pirate-themed web program called *Activate*, comprised adaptive computer games previously shown to improve scholastic performance in children with impulse-control impairments as well as reduce depressive symptoms and executive dysfunction in patients with geriatric depression. Participants in the *Activate* intervention completed a series of games designed to improve functions such as working memory, attentional control, pattern completion, and category sorting. The control program comprised non-adaptive computer games with no unifying theme (Sudoku and single n-back working memory exercises), which have little evidence for improving cognitive function, despite popular conceptions. We further randomized half of the participants in each group to receive information implying either high or low effectiveness of cognitive training, to manipulate their expectations of outcomes at the outset of the program. The intervention was feasible in healthy older adults, with 97% of participants completing the program. Moreover, participants provided positive feedback and reported that they would recommend the program, regardless of experimental condition (75/85 = 88%; $X^2 = 2.59$, *ns*). Analyses of performance on *Activate* revealed a trend towards greater improvement over the course of training by participants assigned to the high expectations subgroup; this trend reached significance in one of the four game types ($F_{(4, 152)} = 3.54$, $p = 0.009$, $\eta^2 = 0.085$). In contrast, we found little difference in the training patterns of those in the high versus low expectations control group. Our results suggest that the present cognitive training intervention is feasible, engaging, and enjoyable, but that high expectations at the outset may facilitate greater improvement during the course of training.

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Poster

649. Human Cognition: Aging II

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Topic: H.02. Human Cognition and Behavior

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Title: Age-related changes in dopamine reduce cognitive flexibility by disrupting fronto-striatal-thalamic functional connectivity

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Abstract: Aging is associated with reduced cognitive flexibility and dysregulation of dopamine, including elevated dopamine synthesis capacity in dorsal caudate nucleus (DCA) relative to young adults measured by 6[^{18}F]fluoro-L-*m*-tyrosine (FMT) PET. FMT is a measure of dopamine synthesis capacity similar to FDOPA, and is quantified as net tracer influx (K_i). Our previous research in young and older adults revealed an inverted U-shaped relationship between cognitive flexibility and DCA FMT K_i, such that extreme high K_i in older adults and extreme low K_i in young adults was associated with reduced flexibility indexed as larger behavioral switch cost. The influence of DCA dopamine on switch cost may be mediated by its modulation of activity within the fronto-striatal-thalamic loops supporting cognitive flexibility. Here we tested the hypothesis that non-optimal DCA FMT K_i is associated with disrupted functional connectivity during task switching in young (n = 21, mean age = 23.8) and older (n = 15, mean age = 77.4) adults. Specifically, we used fMRI and psychophysiological interaction analyses to measure task-related increases in DCA connectivity during switch trials, and examined its relationship with switch cost and DCA FMT K_i. *A priori* region of interest analyses revealed that stronger task-related connectivity between DCA and left inferior frontal gyrus was related to smaller switch cost ($r = -.45$, $p < .01$). Evaluation of the relationship between dopamine and connectivity strength revealed a significant FMT x connectivity x age interaction ($F(1,32) = 4.82$, $p < .05$) such that high K_i in the older adult group and low K_i in the young adult group was associated with reduced fronto-DCA connectivity. Complementing these findings, exploratory voxel-wise regression analysis revealed that increased DCA-thalamic connectivity was also related to smaller switch cost. Likewise, analysis of DCA-thalamic connectivity demonstrated a significant FMT x connectivity x age interaction ($F(1,32) = 5.62$, $p < .05$) such that high K_i in older adults and low K_i in young adults was associated with reduced DCA-thalamic connectivity. Together, these data provide evidence that cognitive flexibility is supported by fronto-striatal-thalamic connectivity, and that connectivity within this circuit is acutely sensitive to DCA dopamine function. We suggest that age-related declines in flexibility are mediated by the effect of dysregulated dopamine on the tuning of striatal circuits.

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Poster

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Title: Multitasking of young and older adults in ecologically valid scenarios- the association with executive functions

Authors: ***C. JANOUCH**[1], U. DRESCHER[2], O. L. BOCK[2], C. VOELCKER-REHAGE[1]; [1]Sports Psychology (Rehabilitation and Prevention), Univ. of Technol. Chemnitz, Chemnitz, Germany; [2]German Sports Univ. Cologne, Cologne, Germany

Abstract: Many daily and sports activities rely on our ability to perform multiple motor and cognitive tasks concurrently, e.g., when driving a car while using the car's navigation system. Multitasking skills have been shown to decrease with advancing age (e.g., Verhaegen et al., 2003). The underlying mechanisms and interplay between cognitive and motor decline are, however, still unclear. It is proposed that executive functions (EF) might contribute towards multitasking, particularly in old age (Baddeley, 1986). Thus, first, we investigated the association between the partitioned EF systems' components (task switching, memory updating, response inhibition (Miyake et al., 2000) and dual-tasking (Strobach et al., 2014)) and the performance in multitasking situations. Moreover, performance in laboratory settings seems to differ from performance in everyday life (Bock & Züll, 2013). Thus, we further aimed to investigate age-related differences in multitasking performance in realistic (virtual) scenarios, namely driving and street crossing. Therefore, executive functions of young (20-30 years) and older (65-75 years) adults are assessed with standardized laboratory tests (updating: spatial n-back task; inhibition: Simon task; task shifting: Geometric figure task; dual-tasking: visual tracking task with tone recognition). Multitasking skills in natural scenarios are assessed by use of two virtual-reality (VR) tasks (simulated street crossing and car driving) combined with a battery of realistic (cognitive) loading tasks that draw on participants' working memory, reasoning and manual skills. Data are currently collected. We expect typical age-related differences in multitasking performance between young and older adults. Age-related differences in the influence of EF on multitasking performance (street crossing and driving task) are analyzed by regression analysis. We expect a moderate effect of EF on multitasking scores in the two VR scenarios. This effect should be stronger for older than for young participants, due to the age-related increase of cross-domain associations (Baltes & Lindenberger, 1997). The understanding of age-related decline in multitasking and the potential contribution of EFs will contribute to develop optimal training programs to improve multitasking performance, particularly in older adults.

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Poster

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Title: Body mass index is associated with white matter changes and executive functions among healthy elderly

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Abstract: Obesity is often discussed as being associated with primary and secondary changes in brain structure and cognition. However, previous studies yielded controversial results, especially with regard to white matter (WM) changes that might be due to differences in sample size, age, sex, and body mass index (BMI) between studies. Therefore, we aimed to assess the effect of BMI on WM microstructure and cognition using diffusion tensor imaging (DTI) in a large, well-characterized population-based sample of healthy older adults.
Analyses were conducted in 586 participants of the LIFE-cohort in Leipzig, Germany (48% females, 60-80 years, BMI 16.81-38.56 kg/m^2) who underwent DTI neuroimaging at 3T and neurocognitive testing. Voxelwise statistical analysis of fractional anisotropy (FA) as a measure of WM fiber tract orientation and density, was carried out using Tract-Based Spatial Statistics (TBSS) implemented in FSL (www.fmrib.ox.ac.uk/fsl/). Associations between BMI and WM tracts were conducted based on permutation tests adjusting for confounders (age, sex and WM hyperintensities). Potential associations with cognitive performance were assessed using partial correlations and mediation analyses controlled for age, sex, and education.
A significant negative correlation between higher BMI and lower FA was observed in

widespread areas including the entire corpus callosum (CC), corona radiata (CR) and internal capsule (IC) ($p<0.05$, FWE-corrected). FA of a BMI-associated cluster in the anterior CC was negatively correlated with BMI ($r=-0.205$, $p<0.01$), whereas it was positively correlated with executive functions ($r=0.101$, $p<0.05$). On the other hand, a higher BMI was correlated with lower scores of executive functions ($r=-0.089$, $p<0.05$). Preliminary mediation results indicated that lower executive functions would translate into lower FA via increasing BMI ($ab = 0.019$, $|BBCI| > 0$), and that BMI would again have a negative contribution to executive functions via its negative effect on FA ($ab = -0.019$, $|BBCI| > 0$).
In this large cross-sectional study we found that obesity, independent from confunders, was associated with reduced WM fiber tract orientation or density in areas including the CC, CR, and IC in healthy elderly individuals. Moreover, our findings indicate a possible role for WM dysfunction in a presumably vicious circle of lower executive functions contributing to higher BMI which in turn provokes regional WM changes, which again contribute to poorer executive functions. Future longitudinal studies are needed to further support this hypothesis and to identify possible underlying mechanisms.

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Poster

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Title: Comt val158met polymorphism is associated with decreased cortical thickness for val carriers in regions of the dopaminergic system

Authors: ***G. G. MIRANDA**, K. M. RODRIGUE, J. R. RIECK, K. M. KENNEDY;
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Abstract: Aging is associated with regionally specific decreases in cortical thickness and subcortical brain volume in healthy individuals. Cognitive aging and to a lesser extent, structural

brain aging have been demonstrated to be partly under genetic control. One common specific genetic polymorphism frequently associated with cognitive performance is the COMT Val158Met polymorphism (COMT). Individuals with the Val/Val genotype demonstrate lower prefrontal dopamine availability coupled with less efficient neural processing. Given the effects of age on brain structure in the dopaminergic pathways, we investigated the influence of COMT polymorphism on cortical thickness and subcortical volume in *a priori* determined regions of interest (ROIs) in frontal, parietal, cingulate, and subcortical areas. We further tested the resource modulation hypothesis, whereby aging is hypothesized to magnify modulation of genetic effects on brain integrity. Our sample consisted of 158 healthy participants across the lifespan for which MRI and COMT genotype data were collected (aged 20-94; Met/Met *n*=38, Val/Met *n*=77, Val/Val *n*=43). FreeSurfer pipeline with manual edits was used to extract cortical thickness and subcortical volume measures. Results indicated significant main effects of COMT genotype beyond the effects of age: we observed significantly thinner cortex for the Val/Val compared to Met/Met group in dorsolateral prefrontal cortex (inferior frontal, middle frontal, superior frontal gyri). Additionally, posterior (but not anterior) cingulate gyrus was thinner for the Val/Met compared to Met/Met group. We found no effect of COMT for orbitofrontal or parietal regions. Effects of COMT on subcortical structures ranged from small but significant (caudate, putamen) to trend-level (accumbens). Thus, individuals with suspected lower prefrontal dopamine availability (Val carriers), have thinner cortex and smaller subcortical structures selectively in regions associated with dopaminergic pathways in the human brain. Contrasting the resource modulation hypothesis, in our lifespan sample, we found no magnified modulation of brain integrity with age (i.e., Age x COMT interactions); we find the effect of COMT to be present at any point in the adult lifespan, and not limited to elderly populations.

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Poster

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Title: Age-related dual task-interference in visual perception while walking, standing, and sitting

Authors: J. PROTZAK, *K. GRAMANN;
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Abstract: With advanced age, once largely automated movements like simple walking can become a challenging task that requires increased attention. Age-related deficits in mobility and sensory processing have to be corrected and possibly compensated by enhanced concentration and cognitive control. But even if an compensatory strategies lead to a more stable gait, the necessary cognitive resources might not be available. Thus, efficient and safe movement execution is more likely to interfere with simultaneously executed secondary tasks competing for cognitive resources. Little is known about the neural processes that are associated with the cognitive demands resulting from gait and posture and how these influences develop throughout adulthood. This is due to the limitations of traditional brain imaging techniques and the involved difficulties to record data in realistic test situations that include human locomotion. However, recent Mobile Brain-Body Imaging (MoBI) approaches provide feasible techniques to measure and analyze electroencephalography (EEG-) data recorded during normal movement executions. The present MoBI-study was conducted to identify and model the brain dynamics processes accompanying compensation of age-related declines in mobility and sensory processing during dual-task walking. A dual task methodology was used to measure performances variations in a visual detection task and to analyze the accompanying neural processes across three different primary motor task conditions. Two groups of younger (n = 15; age range: 19 - 31 years) and older (n = 15; age range: 69 - 80 years) adults participated in three different motor task set-ups: siting, walking, standing. They were asked to manually respond to randomly presented short visual stimuli from three different peripheral presentation angels (20°, 40° and 60° from the central field of view). EEG-data were recorded continuously with a 64-channel mobile set-up. A standard stimulus-locked event-related potential (ERP) analysis revealed two main findings. Differences in the P300-characteristic for the variation of the visual stimulation angle and motor task condition were found for both age groups. In addition, age-dependent variations in the P300 feature became prominent. These results implicate an age-related modulation of visual perception processes that vary systematically with the difficulty in the primary motor task.

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Poster

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Topic: H.02. Human Cognition and Behavior

Title: Age-related differences in manual dexterity and their association with executive functions, grip strength, and finger tapping.

Authors: *O. VASYLENKO, C. RODRIGUEZ-ARANDA;
Univ. of Tromsø, Tromso, Norway

Abstract: Aging is associated with declines in cognitive and motor functions, among these, declines in manual dexterity are central as dexterity is one of the most important skills in daily activities. Detailed kinematic analyses of age-related differences in dexterity are scarce and a better understanding of the contribution of cognitive functions to its decline is needed.
Objectives: a) to describe age-related kinematic differences in dexterity performance; b) to assess the relationship between dexterity, cognition, and neuromuscular hand function.
Methods: Participants were 45 young and 55 older adults, all right-handed. Dexterity performance was assessed with two subtasks of the Purdue Pegboard Test: inserting pins with the right hand, and inserting pins with the left hand. Movements analyzed were reaching, grasping, transporting, and inserting. The kinematic measures for each movement were path length, mean and peak linear and angular velocities, angles, and their coefficients of variability. The neuropsychological tests were Stroop Color-Word Test, Trail Making Test, Logical Memory, Digit Span, Grip Strength, and Finger Tapping Test.
Results: In the right-hand task older adults had lower angular and linear velocities and lower variability in angular and linear velocities during grasping and transport, but higher linear and angular velocities and their variability during inserting. The left-hand task results were similar, but in addition, older adults had lower linear and angular velocities and more variability in velocities during reaching, as well as larger angle and longer path length during transport. The largest differences were in path lengths during grasping and inserting, with older adults showing considerably longer path lengths in both tasks. Grasping path lengths in both tasks were significantly predicted by tests of executive function, grip strength, and finger tapping speed.
Conclusions: The more pronounced kinematic differences in the left-hand task indicate that even the relatively simple task of inserting pins is more difficult for older adults when performed with the non-dominant hand. Compared to the young group, older adults were slower in all movements except inserting. Higher velocities in the older group during inserting might be due to making more corrective movements. Older adults were also less precise in all movements, as indicated by longer path lengths. Executive function, grip strength and finger tapping were significant predictors of precision during grasping.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant 1R01AG039103

Title: Relationship between episodic memory performance, fMRI correlates of encoding and retrieval and estimates of regional brain structure in older adults

Authors: *M. JAYAKUMAR, M. DE CHASTELAINE, B. E. DONLEY, K. M. KENNEDY, M. D. RUGG;
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Abstract: Episodic memory performance declines with age, as do estimates of regional brain structures. Using fMRI, we have previously reported that encoding-related functional activity in the inferior frontal gyrus (IFG) and hippocampus, and retrieval-related activity in the hippocampus, predict associative memory performance across a sample of older individuals (N=62, aged ca. 65-75 yrs.). Here we examined whether structural measures of these same brain regions explain additional variance in memory performance. Participants were scanned both while they encoded a series of word pairs and, subsequently, while performing an associative recognition task (discriminating between 'intact', 'rearranged', and 'new' pairs). Associative recognition accuracy (pR) was indexed by the difference between the proportions of intact pairs judged intact, and rearranged pairs wrongly judged intact. fMRI subsequent memory effects (intact study pairs later judged intact > intact pairs later wrongly judged rearranged) were estimated for bilateral IFG and left hippocampus, and fMRI recollection effects (intact test pairs judged intact > intact pairs judged rearranged) were obtained for bilateral hippocampus. Volume and thickness of the IFG were obtained by a semi-automated method, whereas hippocampal volumes were measured manually. Together, the three functional measures accounted for approximately 33% of the variance in pR across the 62 older subjects (adjusted $R^2 = .337$, $p < .001$). Including hippocampal volume and either left IFG thickness or volume to the regression model did not improve its fit. By contrast, each of these measures of the right IFG significantly improved the fit of the model, but without reducing the contributions of the functional variables (e.g. for right IFG thickness, adjusted $R^2 = .413$). Whereas none of the functional variables, or hippocampal volume, varied according to age (maximum $r = -.163$, n.s., controlling for performance), left and right IFG volume and thickness were highly age-sensitive (minimum $r = -.535$, $p < .001$). These findings indicate that functional and structural measures can dissociate with respect to their sensitivity to individual differences in memory performance and chronological age. This research was funded by NIH Grant 1R01AG039103.

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Poster

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National Institute for Health Research (NIHR) Oxford Biomedical Research Centre based at Oxford University Hospitals NHS Trust and University of Oxford

Title: Individual differences in the neural mechanisms of superior cognitive ageing: diffusion imaging and longitudinal cognitive findings

Authors: ***C. O'DONOGHUE**[1,2], N. FILIPPINI[3], E. ZSOLDOS[2], S. SURI[2,1], M. KIVIMAKI[4], A. SINGH-MANOUX[4], K. EBMEIER[2], C. MACKAY[1,2];
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Abstract: Whilst cognitive decline is common in old age, some older adults retain intact cognitive abilities, but the neural basis of this is unclear. We report preliminary direct evidence for individual differences in the neural mechanisms of superior cognitive ageing. Using literature-based brain metrics in a data-driven approach, individuals were classified as *maintainers* ('youthful' brain structure & function), *adapters* (reduced structure & increased function), or *decliners* (reduced structure & function) relative to younger adults. Cross-sectional (CS) cognitive data were compared between classified groups. Longitudinal (L) cognitive data were analysed to test whether CS differences in cognition reflect different rates of decline, or lifelong differences between groups. Group differences in diffusion (DTI) imaging measures of white matter (WM) integrity were tested, as intact WM may allow *adapters* to recruit additional functional resources.
Methods: 354 healthy older adults from the Whitehall II imaging sub-study, with L cognitive data from 1997-2013, recently completed an MRI scan (T1, DTI, fMRI) and further CS cognitive

tests. *Classification:* For each subject, grey matter (GM) volume and resting state functional connectivity (FC) were extracted from regions affected by age in an independent sample of old vs. young adults (27 vs. 27), and classified relative to the mean and standard deviation GM and FC in young adults, resulting in 118 maintainers, 56 adapters, and 47 decliners. *Cognition:* CS and L cognition was compared between groups using ANOVA. *DTI:* Fractional anisotropy (FA) maps were compared between groups using tract-based spatial statistics (FWE-corrected at $p<0.05$).
Results: *Cognition:* CS performance was significantly better in both *maintainers* and *adapters*, vs. *decliners*. Decline was observed on all L measures, except vocabulary, but there were no between group differences in amount of decline. *Decliners* performed worse across L time points on short term memory and MMSE. *DTI:* There were no group differences in FA after thresholding.
Conclusion: Better cognitive scores in *maintainers* and *adapters* suggest some cognitively superior older adults may adapt to structural decline by engaging additional functional resources, whilst others maintain relatively 'youthful' brains, although no group difference in cognitive decline suggests *decliners* poorer performance may reflect a lifelong difference, rather than a different trajectory of decline. Statistical modelling may provide more sensitive estimates of cognitive trajectories for these groups. Analysis of other WM metrics (e.g. mean diffusivity) may expand on current findings.

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Poster

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Title: Mindreading from the eyes is diminished in aging but independent from gender - Results from a large population-based study

Authors: *J. KYNAST[1], E. M. QUINQUE[2], A. VILLRINGER[1], M. L. SCHROETER[1]; [1]Neurol., Max-Planck Inst. For Human Cognitive and Brain, Leipzig, Germany; [2]Clin. for Psychiatry, Psychotherapy and Psychosomatic Med., Univ. Hosp. Halle, Halle (Saale), Germany

Abstract: *Background:* Theory of Mind (ToM) is an essential human quality that enables successful social interactions. The perception of social cues and their relation to another person's mental state enables individuals to predict behavior and react properly. As affective information is conveyed through eye gaze, the Reading the Mind in the Eyes test (RMET) is a frequently used tool for the assessment of affective ToM. This is the first study to investigate the effects of gender on a wide age range (19-79 years) in a large, population-based German sample. Further, we investigated the effects of item characteristics on RMET performance.
Methods: The sample comprised 1,324 participants of the LIFE study. We investigated the effect of age (12 groups) and gender (2 groups) on RMET performance by applying ANCOVA and correcting for the effect of verbal abilities. Single comparisons were calculated post-hoc. Furthermore, we investigated if gender characteristics of the RMET items (male/female) modulate mindreading ability in men and women in different age ranges (12 groups) using ANCOVA correcting for verbal abilities. Post-hoc single comparisons were calculated.
Results: Male and female performance did not significantly differ, but young adults performed significantly better than older age groups. Men and women identified mental states significantly better from female eyes as compared to male eyes. This gap becomes larger for older age groups.
Discussion: Men are as proficient as women in mindreading from the eyes. Our data suggests that affective ToM declines with increasing age, which is true for both sexes. Older adults may have problems inferring complex emotional content from limited social cues, especially in the presence of male eye gaze.

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Poster

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Title: White matter microstructure mediates the negative effects of amyloid on default mode network function and executive performance

Authors: *C. BROWN[1], F. A. SCHMITT[2], C. D. SMITH[2], B. T. GOLD[1];
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Abstract: Executive function declines in aging have been associated with declines in default mode network (DMN) function. However, the mechanisms underlying these declines may include declines in white matter (WM) microstructure associated with normal aging and/or increasing amyloid pathology associated with preclinical Alzheimer's disease (AD). In the present study we investigated whether these mechanisms have independent or synergistic effects on DMN function and executive performance.

35 cognitively normal older adults (ages 65-93) and 29 younger adults (ages 18-34) underwent resting-state (rs-) fMRI, task (t-) fMRI, and diffusion tensor imaging (DTI). Older adults also had a lumbar draw of cerebrospinal fluid, from which $A\beta_{42}$ concentration was measured. A working memory task was used for t-fMRI. The DMN was identified in rs- and t-fMRI using independent component analysis. These components were Z-scored and combined to identify voxels showing both peak connectivity at rest and deactivation during the task. Functional connectivity at rest and deactivation during the task were measured within common regions-of-interest (ROIs) formed surrounding these peak voxels. In addition, the same ROIs were used as seeds for probabilistic tractography to identify WM pathways connecting DMN regions. Fractional anisotropy (FA) was measured within DMN WM pathways.

T-tests revealed age-related declines in task performance, DMN function, and FA. Partial correlations showed that both DMN deactivation magnitude and FA in DMN WM were negatively correlated with RT across the entire sample (r = -.39, -.40; $p \leq .002$) and within older adults (r = -.48, -.49; $p \leq .006$), after controlling for age, sex, and education. Further partial correlations showed that DMN deactivation magnitude and FA were positively correlated across the entire sample (r =.26, p = .043) and within older adults (r =.36, p =.045). Within older adults $A\beta_{42}$ was negatively correlated with RT (r = -.45, p =.01) and positively correlated with DMN deactivation magnitude (r =.35, p =.049) and FA (r =.35, p =.049) after controlling for age, sex, and education. Rs-connectivity was not related to any of the other measures across or within groups. Mediation models within older adults revealed that the effect of $A\beta_{42}$ on DMN deactivation magnitude was mediated by lower FA in DMN WM. In addition, the effect of $A\beta_{42}$ on RT was mediated by lower DMN FA and deactivation magnitude.

These findings provide the first evidence that the negative effects of $A\beta_{42}$ on DMN function and executive performance are driven by DMN WM microstructure declines. Interventions aimed at protecting DMN WM microstructure may aid in maintaining executive performance.

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Poster

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Title: Age-related changes in case marker processing: ERP evidence from a verb-final language

Authors: J. SUNG, B. EOM, S. OH, *S. JUN;
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Abstract: Previous research have demonstrated inconsistent evidence on whether aging-related decline emerges in online sentence processing. The current study investigated age-related changes in case marker processing of a verb-final language using event-related potential (ERP) paradigm. Korean is a verb-final language with case markers, which permits a relatively free word order as far as the verb retains in a sentence-final position. Sixteen younger adults (*mean age*=25.8, *SD*=3.1) and fifteen older adults (*mean age*=73.0, *SD*=6.0) participated in the study. Sentence stimuli consisted of plausible and case-marker violation conditions with 30 items per each. Case marker assignment was violated in the second noun phrase. Each phrase was presented for 700ms using a Rapid Serial Visual Presentation paradigm with a 200ms inter-stimulus interval during the 32-ch EEG recording. Greater negativity effects were observed in case marker violation across the groups at 300-500ms. Younger groups presented significantly stronger positivity effects at 700-900ms in the case marker violation condition, whereas the positivity effects were attenuated in elderly adults. The results suggested that Korean speakers may process a syntactic component of a case marker under the semantic frame integration, eliciting the negativity effects associated with semantic violations. Elderly adults showed attenuated effects compared to the young group, indicating age-related changes emerged during real-time sentence processing.

Disclosures: **J. Sung:** None. **B. Eom:** None. **S. Oh:** None. **S. Jun:** None.

Poster

649. Human Cognition: Aging II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 649.24/NNN17

Topic: H.02. Human Cognition and Behavior

Support: MRC Grant: G1001354

Title: Relating mobility to brain structure and function in older adults: A magnetic resonance imaging study of members of the Whitehall II cohort

Authors: *N. DEMNITZ[1], E. ZSOLDOS[2], A. MAHMOOD[3], N. FILIPPINI[2], A. SINGH-MANOUX[4], M. KIVIMAKI[4], C. MACKAY[2], H. DAWES[5], H. JOHANSEN-BERG[1], K. P. EBMEIER[2], C. E. SEXTON[1];
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Abstract: Mobility is a strong predictor of institutionalisations, falls, quality of life and mortality in older adults. More recently, mobility has also been linked to cognitive functioning. The association between mobility measures and brain structure, however, remains under-explored. To investigate the relationship between mobility and the ageing brain, we examined the relations between common mobility measures, cognitive functioning, and MRI measures of brain structure (white matter integrity and gray matter volumes).
The sample (n = 387) was drawn from the on-going Whitehall II Imaging Sub-Study. Participants (mean age 69, SD 5.11; 19% women) had no history of neurological illnesses. Mobility assessments (gait speed, balance test, chair stands) were conducted in 2007-2009. On a subsequent phase of data collection (2012-2015), participants underwent a 3T MRI brain scan and completed a battery of cognitive tests assessing memory, executive function, and processing speed. To examine white matter integrity, voxelwise analysis of fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD), and mean diffusivity (MD) data was carried out using Tract Based Spatial Statistics (TBSS). Permutation-based methods for non-parametric testing were employed for all analyses.
Results showed that older adults with better mobility had better cognitive functioning, and this relationship was evident across domains of mobility (gait speed, balance, chair stands) and cognition (executive function, memory and processing speed). Analyses of global DTI metrics showed that poorer mobility, as measured by the balance and chair stand tests, was associated with higher global AD (balance $p = 0.001$; chair stands $p = 0.043$) and RD (balance $p = 0.001$; chair stands $p = 0.043$). Only the balance test revealed a significant association with FA ($p = 0.005$). No association was found between gait speed and global DTI metrics, or between global

gray matter volume and mobility measures. In voxel-wise analyses, significant voxels were found to be widespread across the white matter. Our findings highlight the strong relationship between mobility and cognition and suggest a global decline in white matter is associated with ageing-related decline in mobility.

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Poster

649. Human Cognition: Aging II

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant K18 AG048706

OSU College of Veterinary Medicine

Title: Age-related differences in spatial memory formation and neural activations in a new virtual Morris water maze task

Authors: J. Y. ZHONG[1], *K. R. MAGNUSSON[3], M. E. SWARTS[2], C. A. CLENDINEN[1], N. REYNOLDS[3], S. D. MOFFAT[1];
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Abstract: This study applied a new virtual rendition of the Morris water maze task, which is commonly used for assessing spatial memory performance in rodents, to an examination of age-related differences in spatial memory among younger (18-30 years) and older (> 60 years) human participants. All participants were males, in correspondence with rodent studies done in the Magnusson lab. Unlike previous studies which used dependent navigational performance measures such as time spent searching for target, distance to reach target (i.e., pathlength), number of path crossings of common *xy* coordinates, and heading errors, this study was the first human study to employ a cumulative proximity to goal measure that corrected for start position using an ideal proximity length across 44 learning trials.
After controlling for age differences in the mean pathlength taken to reach the target in visible platform (control) trials, we found that older adults exhibited greater cumulative proximity to goal (i.e., more searches done away from the target platform) than younger adults. This

difference was most prominent during the first 24 place trials in which participants were uninformed that the hidden platform was fixed on every trial. The two age groups performed equally well across 19 control trials in which they moved directly to a visible platform on every trial, suggesting that age group effects detected from place trials was not confounded by age differences in visuomotor control. Importantly, older adults exhibited a learning curve that plateaued early in the second half of the learning trials (place trials 13 to 24), with significantly higher corrected cumulative proximity than younger adults in the earlier phase of these trials. These behavioral findings suggest that older adults may be poorer at accessing their newly acquired spatial memory for finding a place they reached previously in the absence of explicit knowledge of the stationary nature of that location.
All adults were subsequently informed about the stability of the hidden platform and performed the task in the fMRI scanner across 16 trials. The group-level [place - control] contrast (p = .005, uncorrected) showed that younger adults exhibited higher activation in the left middle frontal gyrus and right caudate, as well as lower activation in the anterior cingulate than poorer performers among the older adults (i.e., below the 50th percentile). The higher frontal activation implicates that younger adults may be better at planning or executing their search behavior while the lower anterior cingulate activation implicates that younger adults may expend lesser attentional resources when finding a known target.

Disclosures: J.Y. Zhong: None. **K.R. Magnusson:** None. **M.E. Swarts:** None. **C.A. Clendinen:** None. **N. Reynolds:** None. **S.D. Moffat:** None.

Poster

649. Human Cognition: Aging II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 649.26/NNN19

Topic: H.02. Human Cognition and Behavior

Title: Cognitive impairment in elderly Mexicans and their association with anemia and hipozinquemia

Authors: *H. A. RUBIO-ZAPATA[1], E. SANCHEZ-AGUILAR[2], P. AGUILAR-ALONSO[3]; [1]Med. School- UADY, Merida, Mexico; [2]Sch. of Medicina, University of Yucatan, Mexico; [3]Facultad de Quimica, BUAP, Puebla, Mexico

Abstract: Cognitive impairment (CI) is a multifactorial process. The aging process is one of its most important risk factors, especially when associated with other pathophysiological conditions such as nutritional deficiencies. Iron and zinc are necessary metals in various metabolic pathways. There are reports their deficiencies associated with cognitive impairments. The aim of

the study was to determine the association between the CI and the presence of anemia and hipozinquemia in older Mexican adults. The study included 187 adults aged 65 or older, from the rural areas of southeastern Mexico (Yucatan). All people with depressive symptoms according Yesavage test were removed (n=21). For CI, we used the Minimum Mental State Examination (Minimental Test). All participants were asked to donate 5 mL peripheral blood to determine the hematocrit and hemoglobin by HemoCue® automated system, the determination of serum zinc was by the colorimetric method of Johnson et al. Chi2 statistical analysis, OR, 95% CI $p < 0.05$. All procedures were performed in compliance with the ethical standards and the School of Medicine of the University of Yucatan authorized the project. Results: 23% of the population were man and 77% women. 39.6% of the population had CI (32% mild 5% moderate, 2.6% severe); 35.4% of the population had anemia and 20% hipozinquemia. CI increased proportionally with age. There was no relation between anemia and hipozinquemia with age. The association between anemia and CI was significant ($p < 0.001$, OR 3.9, IC 1.6-8.9), while the hipozinquemia it was not ($p = 0.17$). Other variables that were associated with the CI were being female, engage in housework or fieldwork, illiterate, widowed and have metabolic and cardiovascular comorbidities. In older Mexican adults, living in rural regions the presence of anemia was associated with cognitive impairment, so it is essential the diagnosis and treat this condition to prevent or halt its progression and improve quality of life of the elderly.

Disclosures: H.A. Rubio-Zapata: None. **E. Sanchez-Aguilar:** None. **P. Aguilar-Alonso:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: Universidad de Guanajuato.

Secretaria de Salud del Estado de Guanajuato.

DIF Gerontológico, León.

Title: Subjective memory complaints associated with depression and anxiety in older women

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Abstract: Introduction: Subjective Memory Complaints (SMC) are everyday forgetfulness which may cause limitations and low quality of life. These have been associated with age, cognitive impairment and Alzheimer. Older women may present symptoms of anxiety and depression as characteristic of aging. However it is not exactly known whether the subjective memory complaints are associated with the emotional state in older adults. The aim was to evaluate the association between subjective memory complaints and emotional state in older women. **Method**: 95 women participated from 60 to 80 years without chronic degenerative diseases. Were applied the Mini-Mental State Examination as a screening test of cognitive status and a questionnaire of subjective memory complaints to assess the frequency of complaints categorized in self-perception, semantic, episodic and working memory. We also applied Depression Inventory Beck to assess depressive symptoms and Trait Anxiety Inventory-State to assess anxiety. Data were analyzed using X^2, crosstabs and the relative risk was obtained. **Results**: The self-perception of changes in memory was significantly associated with depressive symptoms (X^2=10.05, p=0.01, RR=1.36) and anxiety (X^2=8.39, p=0.01, RR= 1.12). Semantic memory is associated with depressive symptoms (X^2=7.89, p=0.04, RR=1.27). Episodic memory is associated with anxiety (X^2=5.02, p=0.02, RR=1.63). Working memory for mathematical calculation was associated with depression ($X^{2=}$16.21, p=0.001, RR=1.26). **Conclusion**: These results indicate that there is more likely of finding symptoms of anxiety and depression in older women with subjective complaints of semantic, working, episodic and self-perception memory.

Disclosures: S.A. Trejo: None. **L. Morado-Crespo:** None. **S. Solis-Ortiz:** None.

Poster

649. Human Cognition: Aging II

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant AG-036818

NIH Grant AG-036848

Title: Multivariate analysis reveals a dynamic relationship between parietal gray matter volume and anterior white matter fractional anisotropy across the healthy lifespan

Authors: *D. A. HOAGEY, J. R. RIECK, K. M. RODRIGUE, K. M. KENNEDY;
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Abstract: Cortical gray matter volume declines across the lifespan, most markedly in prefrontal cortex, but also in parietal and temporal association cortices. White matter integrity also shows differential regional degradation with pronounced decreases in anterior regions followed by moderate decreases in posterior white matter integrity with aging. Few studies examine these structural measures jointly; thus the relative sensitivity to age is unclear. Here we applied the data driven multivariate technique partial least squares (PLS) to examine the relationship between cortical gray matter volume and fractional anisotropy (FA) of white matter with the aim of disambiguating both the spatial and temporal properties (cross-sectional age differences) of structural brain aging. Diffusion and high-resolution T1 weighted MRI were collected from a sample of 188 healthy adults, 20-94 years of age. Gray matter cortical parcellation was performed using Freesurfer, while diffusion tensor calculation and FA maps were created using FSL. Separate data tables for gray matter volume measures and voxel-wise FA were submitted to PLS. Analysis revealed a significant principal component accounting for 82% of total variance in volumetric and FA measures ($p = .0002$). Contributing the most variance to the component was the positive relationship between white matter FA from the anterior corpus callosum, anterior projections of the genu, anterior cingulum, and fornix with gray matter volume in parietal regions. Examination of the latent variables revealed that the relationship between gray and white matter in these regions is dependent on age, such that white matter integrity decreases linearly across the lifespan, whereas gray matter volume remains relatively preserved until late life. Investigating each parietal parcel shows that within young adults (20-34) and our oldest adults (70-94), individual differences in FA were not associated with differences in volume; however, our middle adult groups (35-54 and 55-69) exhibited significant coupling between FA and volume. These findings suggest that there is a periodicity to structure-structure associations across the lifespan, with weaker dependency during the end of development/young adulthood, stronger interdependency in middle age, and weaker dependency in the oldest-old. These results partly support the last-in, first out framework of aging as frontal and parietal gray and anterior white matter have the most protracted development and are the most vulnerable to aging.

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Poster

650. Schizophrenia: Genetics and Genomics

Location: Halls B-H

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Program#/Poster#: 650.01/NNN22

Topic: H.03. Schizophrenia

Title: Association study of CX3CR1 polymorphisms V249I and T280M and imaging of human brain function

Authors: *M. SAKAI[1,3], H. TAKEUCHI[5], Y. KIKUCHI[3], Z. YU[3,2], C. ONO[3], Y. TAKI[6], R. KAWASHIMA[7], H. TOMITA[1,4];
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Abstract: Microglia has been known to play an important role as remyelination, synapse pruning, memory and learning processes. It is essential to maintain normal brain function as well as condition brain environment. CX3CR1, a 7-transmembrane domain G-protein-coupled receptor, is involved in various inflammatory processes. CX3CR1 is expressed on the surface of various peripheral cells, such as T lymphocyte, macrophage, neutrophil, monocyte, NK cell, CD4 positive cell, CD8 positive T cell and mast cell, while microglia is the only CX3CR1-expressing cell in the brain. Several studies have reported that CX3CR1 plays important roles in pruning of synapses, memories and neurogenesis. It was also reported that decreases of the CX3CR1 expression was associated with the number of synapses in the brain of patients with schizophrenia. Thus, evidence suggests involvement of CX3CR1 into brain function and the pathophysiology of psychiatric diseases. On the other hand, a non-synonymous single nucleotide polymorphism (SNP) of CX3CR1, V249I (rs3732379) and T280M (rs3732378), which are located in the sixth and seventh transmembrane domains of the CX3CR1 protein, respectively. T280M is related to difference in leukocyte functions including adhesion, signaling, and chemotaxis. However, the function of V249I is unclear. These polymorphisms are associated with various peripheral diseases, they may affect brain function as well as susceptibility to psychiatric disorders, and however the associations between this SNP and phenotypes in the brain have not been investigated. The principal aim of this study was to evaluate the effect of CX3CR1 polymorphisms on the structure and function in the human brain. To investigate effect of genetic variations in CX3CR1 on the structure and function of the human brain, we evaluated association between the SNP and MRI findings, including volume of gray and white matters, diffusion tensor imaging (DTI)-based white matter integrity and arterial blood volume of cerebral blood flow (CBF) during rest using arterial spin labeling (ASL), among 1,301 healthy Japanese people. The C allele of the SNP was significantly associated with increased arterial blood volume around the precuneus. There was no major difference between brain structural indicators in any brain areas among genotypes. This finding suggests that CX3CR1-T280M and V249I may affect brain artery structures via interaction between microglia and vascular endothelial cells, and underlie relevant brain functions and susceptibilities to brain diseases.

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Poster

650. Schizophrenia: Genetics and Genomics

Location: Halls B-H

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Program#/Poster#: 650.02/NNN23

Topic: H.03. Schizophrenia

Title: Brain cognitive networks and the related genetic components explored with three-way parallel ICA

Authors: *K. MEZEIVTCH[1], C. L. WRIGHT[2], J. LUI[5], Y. WANG[6], R. ARORA[6], J. H. CALLICOTT[7], V. S. MATTAY[3,8], D. R. WEINBERGER[4], Q. CHEN[2];
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Abstract: Multi-modal data collected for the same subjects have become common in neuroscience research. Simultaneously studying such data may yield new information that could not be observed in analyzing individual data, or enhance the common information across data modalities. In this study, we used three-way Parallel Independent Component Analysis (pICA, Vergara et al, 2014) to incorporate two fMRI tasks and genotype data extracted from the top loci reported by the Psychiatric Genetics Consortium (PGC) for schizophrenia in the same model. Data from two fMRI cognitive tasks and genotype data of 89 SNPs for 187 healthy controls were included in this study. Subjects underwent BOLD fMRI (3T) during a working memory task (NBack) and a cognitive control task (Flanker). Eighty nine SNPs ($SNPs_{pgc2\_sig}$) were selected from 108 top loci from published PGC schizophrenia GWAS study (PGC2 2014), excluding indels and SNPs on Chromosome X.
We found 9 sets of correlated components with significant pairwise correlations after Bonferroni correction. Among them, we found one of the correlated components with likely biological meaning, in which the correlation between NBack and Flanker was 0.2598 ($P_{Bonferroni}$=0.0132), the correlation between NBack and genetic data was 0.3 ($P_{Bonferroni}$=0.004), and the correlation between Flanker and genetic data was 0.27 ($P_{Bonferroni}$=0.008). Significant positively activated regions (Z>2) in NBack include BA9 (DLPFC), BA47 (VLPFC), BA32 (Anterior Cingulate). Significant negatively activated regions (Z<-2) included BA39 (Inferior Parietal). Significant positively activated brain regions (Z>2) in Flanker components included BA9, BA47, and BA32. Significant negatively activated brain regions (Z<-2) in Flanker components included BA22 (Superior Temporal Gyrus), BA13 (Insula), and BA23 (Precuneus). Index SNPs within Schizophrenia risk genes CACNA1C, TCF4, ZNF804A are among the top 5 SNPs ranked by absolute values of ICA weighting scores.

Our results show that when we examined the correlation among NBack, Flanker and genetic data simultaneously, previously identified network patterns found in individual fMRI study are also shown in the top correlated components, especially brain regions involved in both tasks, i.e. involving executive control. Previously found candidate genes for schizophrenia are ranked high based on the contribution to the correlation with fMRI data. Our results support the use of 3-way ICA to identify shared brain networks during cognitive tasks and the associated genetic components with these tasks. Further cross validation is necessary to better interpret the findings.

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Poster

650. Schizophrenia: Genetics and Genomics

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Topic: H.03. Schizophrenia

Support: NIH Grant F32MH102931

Title: Behavior phenotypes in mice: should single strains suffice?

Authors: *L. J. SITTIG[1], P. CARBONETTO[2], K. ENGEL[2], K. KRAUSS[2], C. BARRIOS-CAMACHO[2], A. A. PALMER[1];
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Abstract: Genetically engineered mutations are a valuable tool for exploring genotype-phenotype relationships. Most such studies are carried out using inbred strain backgrounds since this allows comparison of mutant and wild type alleles on an isogenic background. These studies are predicated on the notion that genotype-phenotype relationships measured on a single inbred background will generalize to other species; thus there is an implicit assumption that they would at least generalize to other inbred mouse strains. We directly tested this assumption by examining the effects of null alleles of *Cacna1c* and *Tcf7l2*, which have been robustly implicated in schizophrenia and other complex diseases by human genome-wide association studies (GWAS). We bred C57BL/6J heterozygous males to wild-type females from 30 of the most commonly used inbred strains to generate a balanced set of heterozygous and wild-type coisogenic F_1 offspring (N=723 *Cacna1c*, N=630 *Tcf7l2*). F_1 offspring were tested for widely-used behavioral and physiological phenotypes that model aspects of schizophrenia, bipolar disorder, and depression. We found extremely strong interactions between the mutant alleles and

the different genetic backgrounds. Some F_1s were very sensitive to particular effects of the mutant alleles while others appeared completely resistant. In several cases the mutant alleles increased a given phenotype in one F_1 while decreasing the same phenotype in another. These results do not negate the invaluable contributions of mouse genetics to biomedical science; however, they do make an important contribution to the ongoing discussion of reproducibility. We conclude that genotype-phenotype relationships cannot be reliably inferred by studying a single genetic background. Therefore, we suggest that the effect of mutant alleles should be evaluated on several inbred strain backgrounds whenever possible.

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Poster

650. Schizophrenia: Genetics and Genomics

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Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant Number 25116520)

Title: Alternations of DARPP-32 and Calcineurin protein expressions in the prefrontal cortex and nucleus accumbens of schizophrenia and bipolar disorder and genetic associations with their expressions

Authors: *Y. KUNII[1,3], M. HINO[3], J. MATSUMOTO[3], A. WADA[3,4], A. NAGAOKA[3], S.-I. NIWA[2], Y. YOKOYAMA[5,6], H. NAWA[7], H. TAKAHASHI[6], A. KAKITA[6], H. AKATSU[8,9], Y. HASHIZUME[9], S. YAMAMOTO[9], H. YABE[3];
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Abstract: Background: Dopamine- and cAMP-regulated phosphoprotein of molecular weight 32 kDa (DARPP-32) is a central molecule that incorporates dopaminergic signal transduction into many other neurotransmitter systems, such as a glutamatergic system. Phosphorylated DARPP-32 regulates the physiological activities of various proteins. Calcineurin (CaN) is located downstream of dopaminergic and glutamatergic pathways and inactivates DARPP-32 by dephosphorylation. CaN has also been considered to be related with synaptic plasticity or neural cell apoptosis. Although the signaling pathway that contain DARPP-32 and CaN may be the one of the crucial circuit to the basic understanding of schizophrenia and other psychiatric disorders, there have been only a few postmortem brain studies of DARPP-32 and CaN in psychiatric disorders and these results were inconsistent across the studies.
Methods: In this study, the expression levels of DARPP-32 and CaN protein were measured by ELISA in two brain regions receiving dopaminergic input and implicated in schizophrenia (the prefrontal cortex [PFC], and nucleus accumbens [NAcc]) in 49 postmortem samples from subjects with schizophrenia, bipolar disorder, major depression and normal controls. We also examined the association of these expressions with genetic variants of dopaminergic system associated molecules for 129 SNPs in a much larger set of postmortem samples (85 subjects).
Results: DARPP-32 in PFC was marginally significantly decreased in schizophrenia as compared to controls ($P=0.059$). The expressions of CaN in PFC were significantly increased in schizophrenia ($p=0.02$) and bipolar disorder ($p<0.05$) as compared with normal controls. In NAcc, CaN was markedly increased in bipolar disorder ($p=1.33$ E-15), but was not changed in schizophrenia. We analysed 129 SNPs in the dopaminergic system associated molecules and found that several SNPs including the one which was previously reported to be related with schizophrenia might predict protein expression of CaN in PFC and NAcc.
Conclusion: These alternations in the pathway that includes DARPP-32 and CaN may reflect potential molecular mechanisms underlying the pathogenesis of schizophrenia and bipolar disorder or differences between these two major psychiatric disease.

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Poster

650. Schizophrenia: Genetics and Genomics

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Program#/Poster#: 650.05/NNN26

Topic: H.03. Schizophrenia

Title: Facial profile principal components derived from full Procrustes analyses correlate with schizotypal inventory scores and SAT performance in sex- and side-specific manners

Authors: J. I. PERI[1], E. M. CROSS[2], B. N. KEEHAN[2], A. T. DEPEW[2], B. A. SAMONTE[2], T. M. MILEWSKI[2], P. T. ORR[1,2], *J. CANNON[1,2];
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Abstract: Embryological development of the face and anterior brain occur in synchrony, are often influenced by common genes, and have the potential to be dramatically and characteristically altered by genetic abnormalities or environmental insults. Schizophrenia is thought to be caused by genetic and environmental factors that have the potential to affect the development of the brain and face. Hennesy et al. (2004) found that faces of schizophrenics showed significant morphological and sex-specific differences compared to controls. Using only frontal photographs, we previously observed relationships between facial loci and SAT/SPQ scores (Cannon et al., 2015). Here, we generated principal components (PCs) of profile facial loci in undergraduates and related those to overall and subscale scores of the Schizotypal Personality Questionnaire (SPQ) and Math/Verbal SAT scores. Other basic demographics were also assessed.

Left and right digital profile photographs were taken of undergraduates (N = 110; 74.5% female). The x-y coordinates of 37 loci were measured blind using ImageJ and processed with a full Procrustes superimposition and principal component analyses utilizing SPSS. In each case, left, right, and the average of left and right profiles resulted in 7 PCs with eigenvalues greater than 1 that typically accounted for almost 80% of variance. These PCs, listed in order of average eigenvalue strength, dealt with the following facial structures: Ear, Lips, Eyebrow/Eye, Lower Nose, Chin, Nasion/Brow Ridge, and Jaw/Face Perimeter. The PCs were correlated with the measures listed above.

The following represent the strongest correlations observed. In each case, there was a significant difference between left and right profile correlations (p = .04 to .002). For SPQ, Right Chin showed significant negative correlations in Females and Total Score ($r(66) = -.382$, $p = .002$) as well as 2 of the 3 subscales. Male correlations did not significantly differ and were in the same direction. For SAT Verbal, Left Nasion/Brow Ridge showed a significant negative correlation in Males ($r(14) = -.634$, $p = .015$). Females showed a significantly weaker, near 0, correlation. For SAT Math, Left Lower Nose showed a significant negative correlation in Males ($r(14) = -.692$, $p = .006$). Again, Females showed a significantly weaker, near 0, correlation.

Comparing these results to our earlier examination of only frontal facial landmarks, it seems clear that profile images can provide additional information relevant to both schizotypal traits and SAT Math/Verbal scores.

Disclosures: J.I. Peri: None. **E.M. Cross:** None. **B.N. Keehan:** None. **A.T. DePew:** None. **B.A. Samonte:** None. **T.M. Milewski:** None. **P.T. Orr:** None. **J. Cannon:** None.

Poster

650. Schizophrenia: Genetics and Genomics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 650.06/NNN27

Topic: H.03. Schizophrenia

Title: DISC1 controls calcium transfer from endoplasmic reticulum to mitochondria

Authors: S. J. PARK, S. LEE, B. GOO, *S. PARK;
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Abstract: DISC1 (*Disrupted-in-schizophrenia 1*) has emerged as a convincing susceptibility factor for the major mental illnesses including schizophrenia, recurrent major depression, and bipolar disorders, but its mechanistic links to the diseases are yet to be fully understood. The calcium plays essential roles in diverse and fundamental biological processes of a cell and an organism. This maximized functional variety of calcium is based on its fast effects and its spatially and temporally dynamic properties, which is heavily dependent on the calcium buffering function of organelles such as endoplasmic reticulum (ER) and mitochondria. Here, we identified that DISC1 localizes at the mitochondria-attached endoplasmic reticulum membrane (MAM), a specialized ER-mitochondrial contact site, and influences the calcium transfer from ER to mitochondria which is mediated by inositol-1,4,5-trisphosphate (IP3) receptors. The perturbed regulation of the calcium transfer between two subcellular organelles by DISC1 malfunction induced abnormal calcium accumulation in mitochondria upon stimulation of oxidative stress, thereby impairing mitochondrial functionalities. Our findings give a hint at the mechanistic links between DISC1 function and environmental stimuli with implications in the pathobiology of the related psychiatric conditions.

Disclosures: S.J. Park: None. **S. Lee:** None. **B. Goo:** None. **S. Park:** None.

Poster

650. Schizophrenia: Genetics and Genomics

Location: Halls B-H

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Program#/Poster#: 650.07/NNN28

Topic: H.03. Schizophrenia

Title: Polygenic risk scores for schizophrenia of miR-137 target genes is associated with brain function during working memory

Authors: *Q. CHEN[1], C. L. WRIGHT[1], K. M. MEZEIVTCH[1], J. H. CALLICOTT[2], D. R. WEINBERGER[1,3,4,5,6];
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Abstract: Strong association with schizophrenia has been reported repeatedly within a region containing *MIR137HG*, the host gene encoding microRNA 137 (miR-137). Previous studies suggest that target genes of this microRNA are also involved in many schizophrenia relevant biological pathways. Therefore studying the genes regulated by miR-137 may be important in understanding the etiology of schizophrenia. Collins *et al*., 2014 reported that 420 genes were differentially expressed (DE) ($q<0.05$) at 48h following miR-137 overexpression in a human neural stem line. In our current study, we used NBack, a working memory task, to examine the effect of schizophrenia risk profile score (RPS) of miR137-regulated genes on brain activation. Two hundred and sixty seven healthy volunteers underwent BOLD fMRI (3T) during a working memory task (NBack). RPSs were calculated for each individual as the weighted sum of reference alleles of LD clumped SNPs within the total 420 DE genes (RPS_{all}), and separately within the downregulated (156) and upregulated (264) DE genes (RPS_{up} and RPS_{down}). SNPs within each gene (extended by 2K at each end) were included based on $P < 0.05$ of the PGC2 results. The PGC2 identified risk SNP, rs1702294, within *MIR137HG* was used to test for a stratification effect on the association of RPS with brain function.
Our results show no significant association of RPS_{all} with brain activation during the NBack task. However there is a significant positive correlation of RPS_{down} with IFG activation ($p=0.023$, FDR corrected in IFG) and no significant negative correlation for RPS_{down}. Whereas, there is a trend of negative correlation of RPS_{up} with brain activation in IFG ($p=0.091$, FDR corrected in IFG), but no positive correlation of RPS_{up} with brain activation. When we stratified our sample according to rs1702294 genotype, we found a strong positive correlation between RPS_{down} and IFG activation ($r=0.30$, $p=6.03e-05$) only in homozygous risk alleles carriers, but not in non-risk carriers ($r=0.14$, $p = 0.196$). There was no rs1702294 stratification effect for RPS_{up} in IFG.
Our results show that schizophrenia-associated SNPs among genes downregulated by miR-137 overexpression are associated with brain activation of the inferior frontal gyrus region during a working memory task. This association is driven by homozygous carriers of the rs1702294 risk SNP. From literature, IFG is heavily activated by the Nback and thought to compensate for abnormal dlPFC activation in patients with schizophrenia. Our current finding suggests that the *MIR137HG* gene risk SNP interacts with SNPs among putative downregulated targets of miR-137 in affecting brain function related to schizophrenia.

Disclosures: **Q. Chen:** None. **C.L. Wright:** None. **K.M. Mezeivtch:** None. **J.H. Callicott:** None. **D.R. Weinberger:** None.

Poster

650. Schizophrenia: Genetics and Genomics

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Program#/Poster#: 650.08/NNN29

Topic: H.03. Schizophrenia

Support: NIH R01 MH097803

Title: Regulation of the serotonin 2a receptor gene (htr2a) in the mouse prefrontal cortex by early growth reponse 3 (egr3)

Authors: *X. ZHAO[1], D. REZNIK[2], A. MAPLE[3], A. MCBRIDE[3], J. LISH[3], A. GALLITANO[3]; [1]Interdisciplinary Program in Neurosci., Arizona State Univ., Tempe, AZ; [2]Barrett, the Honors College, Arizona State Univ., Tempe, AZ; [3]Univ. of Arizona Col. of Medicine- Phoenix, Phoenix, AZ

Abstract: The immediate early gene *EGR3* is associated with schizophrenia in humans and expressed at reduced levels in postmortem patients' brains. We previously found that, in addition to schizophrenia-like behavioral abnormalities, *Egr3*-/- mice have a nearly 70% decrease in prefrontal cortical serotonin 2A receptors ($5HT_{2A}$Rs). This underlies their resistance to sedation by clozapine, a phenomenon that parallels the increased tolerance of schizophrenia patients to antipsychotic side effects. These findings led us to hypothesize that EGR3, a transcription factor, directly regulates the expression of the $5HT_{2A}$R - encoding gene *Htr2a*. To test this hypothesis, we collected tissue after 8 hours of sleep deprivation (SD) from *Egr3 -/-* and wildtype (WT) mice that also carried an enhanced green fluorescent protein (EGFP) reporter construct under control of the *Htr2a* promoter (*Htr2a*-EGFP). We used antibodies against EGFP to detect $5HT_{2A}$R-expressing neurons. Our images suggest *Egr3-/-* mice had less EGFP labeling compared to WT mice, particularly in layers 4-5 of the cortex. Using bioinformatics analyses we searched for high probability EGR consensus binding sites in the *Htr2a* promoter. We identified a "distal" site located ~2800 bp upstream of the transcription start site, and a "proximal" site ~70 bp upstream of *Htr2a* transcriptional start site. To determine the expression time-course of EGR3 protein we conducted western blot analysis on prefrontal cortex tissue from WT mice at baseline, compared with animals sacrificed two hours and three hours after electroconvulsive seizure (ECS), which induces maximal expression of EGR3. These results showed that EGR3 protein levels were highest two hours after ECS. Based on these findings we conducted Chromatin Immunoprecipitation (ChIP) on frontal cortical tissue isolated from WT mice two hours following ECS to evaluate whether EGR3 directly binds to these regions of the Htr2a promoter in *vivo*. ChIP revealed significant binding of EGR3 to the distal region in the *Htr2a* promoter after ECS compare with baseline. To confirm functionality of EGR3 binding to the *Htr2a* promoter, we performed an *in vitro* luciferase-reporter assay. Results of these studies will be

presented. These findings provide information about potential regulation of the schizophrenia candidate gene *HTR2A* by the immediate early gene transcription factor EGR3.

Disclosures: X. Zhao: None. **D. Reznik:** None. **A. Maple:** None. **A. McBride:** None. **J. Lish:** None. **A. Gallitano:** None.

Poster

650. Schizophrenia: Genetics and Genomics

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Topic: H.03. Schizophrenia

Support: Estonian Research Counsil grant PUT129

Estonian Research Counsil grant IUT20-41

Title: The expression levels of the genes coding for the IgLON family of neural cell adhesion molecules in the dorsolateral prefrontal cortex of schizophrenic patients

Authors: *M.-A. PHILIPS[1], K. KONGI[1], K. SINGH[1], E. LEIDMAA[2], E. VASAR[1];
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Abstract: Growing evidence suggests that the family of IgLON neural adhesion molecules (OPCML, NTM, LSAMP, NEGR1) are important candidates in forming the susceptibility of schizophrenia. IgLONs have been suggested to be involved in neurite outgrowth, synapse formation and pruning and neurogenesis, all of what has been shown to be altered in the brains of schizophrenic patients. Furthermore, it has been found that the level of the LSAMP protein is approximately 20% increased in the *postmortem* frontal cortex in patients with schizophrenia. According to our recent results, there are certain tag SNP-s in the 1b promoter of the human LSAMP gene that have a strong association with the risk of schizophrenia. LSAMP gene has been in the focus of our behavioral, anatomical, association and cell culture studies in many years. As we have understood the importance of studying IgLON family as a highly regulated molecular complex, we have recently included other IgLON members as our molecular targets. Our aim was to characterize the expression levels of the genes coding for the IgLON family in the dorsomedial prefrontal cortex of schizophrenic patients by using quantitative RT-PCR. Tissue samples were obtained from from Australian Brain Bank Network. Alternative 1a and 1b promoter activities of LSAMP, NTM and OPCML were quantified separately. We found significantly higher expression of NEGR1 and NTM 1b transcript *postmortem* dorsolateral prefrontal cortex in patients with schizophrenia (n=36) compared with control subjects (n= 35).

Significant change in mRNA transcript in NEGR1 was confirmed in protein level by using western blot. Interestingly, it has been previously shown that NEGR1 transcript is significantly increased in dorsolateral prefrontal cortex of human subjects with major depressive disorder compared with non-psychiatric control subjects indicating that changes in NEGR1 levels/activity are underlying of wider spectrum on psychiatrical conditions. Furthermore, according to our preliminary cell culture experiments, NEGR1 is an important mediator in the process of neurite outgrowth. Suprisingly, we found no changes in the levels of LSAMP 1a and 1b which would be expected from previous results. However, our future research will cover wider regions in the brains of schizophrenic patients and controls, we will also continue with cell culture experiments to shed light on the exact functions of IgLON family of neural adhesion molecules and their impact in modifying neuronal substrate underlying in normal and pathological psychological/psychiatric conditions.

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Poster

650. Schizophrenia: Genetics and Genomics

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Program#/Poster#: 650.10/NNN31

Topic: H.03. Schizophrenia

Support: Medical Research Council UK MC_UU_12013/3

Medical Research Council UK G1002064

Elizabeth Blackwell Institute Catalyst Fund

Title: Evidence for impaired motor learning and reduced spindle density in carriers of ZNF804A psychiatric risk alleles.

Authors: *U. BARTSCH[1], L. CORBIN[2], M. TAYLOR[2], C. HELLMICH[3], C. DURANT[4], M. W. JONES[5], N. J. TIMPSON[2];
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Abstract: The rs1344706 single nucleotide polymorphism (SNP) in ZNF804A was among the first genetic variants associated with schizophrenia. Since then a number of studies have examined the influence of rs1344706 on cognition, brain structure and physiology. Despite this, the impact of this SNP on sleep physiology and cognitive aspects of sleep function has not been

described.
We used a recall-by-genotype design implemented in a population cohort (the Avon Longitudinal Study of Parents and Children) to investigate impacts of ZNF804A variants on sleep architecture, neurophysiology and sleep-dependent memory consolidation in healthy young adult homozygote CC (non-risk, n=11) and AA (risk, n=13). We used actigraphy with sleep diaries to assert weekly sleep wake cycles. Participants were monitored using lab-based polysomnography (PSG) on two nights. A motor sequence task (MST) was used to probe sleep-dependent memory consolidation over the second night.
Actigraphy and sleep diaries over the course of 2 weeks revealed similar rest-activity cycles between groups. We found no evidence for differences in actigraphy derived variables such as sleep latency and total sleep time between groups. PSG for both nights was scored by experts and subsequent analysis revealed no evidence for differences in PSG derived sleep latency, total sleep time or time spent in stages REM and NREM1-3 between CC and AA groups on either of the two PSG nights.
In preliminary analyses, there was evidence that the AA group performed reduced numbers of correct sequences (Mann-Whitney P value = 0.026), displayed slower average transition speed times (button press latency, Mann-Whitney P value = 0.046) and greater variance in overnight MST improvement (Levene's test, p=0.050). An automated analysis of non-REM sleep epochs provided evidence for reduced spindle densities (Mann-Whitney P value = 0.060) in AA carriers. These effects of ZNF804A on motor learning and non-REM sleep physiology provide novel insights into the genetic basis of psychiatric disorders.

Disclosures: **U. Bartsch:** A. Employment/Salary (full or part-time): Eli Lilly UK. **L. Corbin:** None. **M. Taylor:** None. **C. Hellmich:** None. **C. Durant:** None. **M.W. Jones:** None. **N.J. Timpson:** None.

Poster

650. Schizophrenia: Genetics and Genomics

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Program#/Poster#: 650.11/NNN32

Topic: H.03. Schizophrenia

Title: Molecular, functional, and pharmacological characterization of M4L, a muscarinic acetylcholine M4 receptor splice variant

Authors: D. A. SCHOBER[1], C. H. CROY[1], C. L. A. RUBLE[1], R. TAO[2], K. DEBROTA[1], *C. C. FELDER[1];

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Abstract: Through bioinformatics, an open reading frame was discovered in the human, mouse and rat with a common ATG (methionine start codon) that extended the N-terminus of the muscarinic acetylcholine M4 receptor subtype by 155 amino acids resulting in a longer variant. RT-PCR experiments performed from human donor brain prefrontal cortex detected the human upstream exon and the junction of the two exons indicating the translation of the mature longer M4 receptor transcript. The predicted size for the longer two-exon M4 receptor splice variant with the additional 155 amino acid N-terminal extension, designated M4L is 69.7 kDa compared to the 53 kDa canonical single exon M4 receptor (M4S). Western blot analysis from a mammalian overexpression system, and saturation radioligand binding with [3H]-NMS (N-methyl-scopolamine) demonstrated the expression of this new splice variant. Comparative pharmacological characterization between the M4L and M4S receptors revealed that both the orthosteric and allosteric binding sites for both receptors were very similar despite the addition of an N-terminal extension. However, when both receptors were coexpressed the M4L appeared to have a dominate-negative effect on the functionality of the canonical receptor which could have implications in M4 receptor biology.

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Poster

650. Schizophrenia: Genetics and Genomics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 650.12/NNN33

Topic: H.03. Schizophrenia

Title: DNA methylation changes in leukocytes by clozapine treatment in patients with treatment-resistant schizophrenia.

Authors: *M. KINOSHITA[1], S. NUMATA[2], A. TAJIMA[3,4], H. YAMAMORI[5], Y. YASUDA[5], M. FUJIMOTO[5], I. IMOTO[3], R. HASHIMOTO[5,6], T. OHMORI[2];
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Abstract: Objective Clozapine is a kind of atypical antipsychotics, which is effective for the treatment-resistant schizophrenia. DNA methylation is a major epigenetic mechanism, and it is affected by environmental factors. In this study, we examined DNA methylation changes by clozapine treatment in the patient with treatment-resistant schizophrenia by conducting a genome-wide DNA methylation profiling (485,764 CpG dinucleotides) of peripheral leukocytes. **Method** Twenty-one patients with schizophrenia (mean age: 42.1 ± 11.4 y; male: 8, female 13) were recruited from Tokushima and Osaka University Hospitals in Japan. The mean dose of clozapine was 473.8 ± 91.3 mg/day, and the mean duration of clozapine treatment was 340.8 ± 182.7 days. Peripheral bloods were collected before and after introduction of clozapine. The psychotic symptoms of patients were evaluated by Positive and Negative Syndrome Scale (PANSS) when the peripheral blood samples were collected. DNA methylation level was assessed with Infinium® HumanMethylation450 BeadChips (Illumina Inc.). A paired t-test was used to see the DNA methylation changes induced by clozapine. A single regression analysis was used to assess the relationship between the DNA methylation changes and the changes of psychotic symptoms evaluated by PANSS. **Result** Of the 435,611 CpG sites, significant DNA methylation changes after clozapine treatment were observed in 21,481 sites ($P < 0.05$ and beta difference > 0.01). Of the 21,481 CpG sites, significant correlations between % PANSS improvements and DNA methylation changes were observed at 414 sites ($p < 0.001$). **Conclusions** Our findings suggest that DNA methylation changes of the specific genes may be associated with treatment response to clozapine in the patients with treatment-resistant schizophrenia.

Disclosures: M. Kinoshita: None. **S. Numata:** None. **A. Tajima:** None. **H. Yamamori:** None. **Y. Yasuda:** None. **M. Fujimoto:** None. **I. Imoto:** None. **R. Hashimoto:** None. **T. Ohmori:** None.

Poster

650. Schizophrenia: Genetics and Genomics

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Program#/Poster#: 650.13/NNN34

Topic: H.03. Schizophrenia

Title: Expression of microRNAs and inflammatory markers in schizophrenia and bipolar disorder

Authors: *S. K. AMOAH[1], B. A. RODRIGUEZ[2], N. MELLIOS[2];
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Abstract: Schizophrenia and bipolar disorder are multifactorial and heterogeneous psychiatric disorders, whose pathogenesis remains elusive, despite the numerous protein-coding genes potentially linked to their pathophysiology. However, the emerging consensus is that protein-coding genes inhabit only the tip of the iceberg of the mammalian transcriptome, given the plethora of actively transcribed non-coding RNAs (ncRNAs). One specific category of small ncRNAs with numerous, yet conflicting reports of aberrant expression in psychiatric disorders is that of the evolutionarily conserved small ncRNAs known as microRNAs (miRNAs). Previous approaches in miRNA profiling were hindered by their inability to appropriately discriminate between precursor and mature miRNA sequences and within members of the same miRNA families. We aimed at employing cutting-edge miRNA methodologies to determine with great accuracy the expression and potential function of schizophrenia-altered miRNAs in the human brain. To that end, we utilized NanoString miRNA expression profiling to specifically screen for differentially expressed mature miRNAs in the orbitofrontal cortex (OFC) of a large cohort of subjects with schizophrenia and bipolar disorder. Validation of mature miRNA levels was carried out for select schizophrenia dysregulated miRNAs using Taqman qRT-PCR assays. We found significant increases in a subset of miRNAs previously linked to immune activation, as well as downregulation of a small number of activity-dependent miRNAs related to synaptic plasticity, with the most prominent being miR-132. Previous studies have suggested a link between neuroinflammatory markers and psychiatric disease. Our data also revealed a significant elevation of inflammatory marker Serpina3 in the brain of subjects with schizophrenia with a trend for increase in bipolar disorder relative to unaffected control samples. Interestingly, there was an inverse relationship between Serpina3 and miR-132 expression and a significant association of both molecules (negative for Serpina3, positive for miR-132) with the age of onset of schizophrenia. Ongoing experiments are examining the developmental expression, downstream targets, and effects on neuronal structure and function of schizophrenia- and Bipolar disorder- altered miRNAs in patient-derived neuronal cultures, as well as mouse models of prenatal inflammation. In doing so, we expect to significantly advance the current understanding of the molecular mechanisms that may underlie psychiatric disorders.

Disclosures: **S.K. Amoah:** None. **B.A. Rodriguez:** None. **N. Mellios:** None.

Poster

650. Schizophrenia: Genetics and Genomics

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Program#/Poster#: 650.14/NNN35

Topic: H.03. Schizophrenia

Support: Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan

Tokyo Medical and Dental University Funds

Title: Genetic association between DLG1 gene and schizophrenia

Authors: *A. UEZATO[1], N. YAMAMOTO[1], D. JITOKU[1], S. HIRAOKA[1], E. HARAMO[1], E. HIRAAKI[1], Y. IWAYAMA[2], T. TOYOTA[2], M. UMINO[1], A. UMINO[1], Y. IWATA[3], K. SUZUKI[3], M. KIKUCHI[4], T. HASHIMOTO[5], N. KANAHARA[5], A. KURUMAJI[1], T. YOSHIKAWA[2], T. NISHIKAWA[1];
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Abstract: The N-methyl-D-aspartate type glutamate receptor (NMDAR) are presumed to be hypoactive in brains of patients with schizophrenia. Several studies have indicated an altered proteins or mRNA expression of one of its scaffolding protein, the discs, large homolog 1 (DLG1), also known as synapse-associated protein 97 or SAP97 in the disease. The *DLG1* gene resides in the chromosomal position 3q29 where an excess of microdeletions in schizophrenia was found in multiple genome-wide analyses on copy number variations. In the present study, we have replicated the genetic association between the *DLG1* gene and schizophrenia shown in our two previous studies, using a Japanese cohort with 1808 cases and 2170 controls. We identified an unreported exon (Exon 3b) in the *DLG1* gene, and detected a significant genotypic and allelic association of the SNP rs3915512 located in the exonic splicing enhancer (ESE) sequence of this exon ($p = 0.005$ (permutation $p = 0.0164$) and 0.0032 ($p = 0.0092$), respectively). When the allele A, and not the schizophrenia risk allele T, of the SNP meets the consensus sequence of ESE, it could lead to the expression of a newly identified *DLG1* splicing variant, whose mRNA expression has been shown to be reduced in postmortem brains of early-onset (before 17) schizophrenia in our previous study. Furthermore, onset age-selective (after 18) associations with schizophrenia have been observed for the SNP. A meta-analysis of the previous and present studies of the SNP rs9843659 revealed its significant association with schizophrenia ($p = 0.033$). These findings add a support to the view that *DLG1* gene could be implicated in the onset age-associated susceptibility and/or pathophysiology of schizophrenia.

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Poster

650. Schizophrenia: Genetics and Genomics

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Program#/Poster#: 650.15/NNN36

Topic: H.03. Schizophrenia

Support: Danish Strategic Research Fund

Title: Epigenetic regulation of human nicotinic acetylcholine receptor gene promoter.

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Abstract: The α7 nicotinic acetylcholine receptor regulates a wide variety of developmental and secretory functions in neural and non-neural tissues. The receptor is now considered a promising target for treatment of cognitive symptoms in psychiatric disorders. By using DNMT inhibitors, it has been found that DNA methylation regulates Chrna7 transcription in several human cell lines. As α7 nicotinic acetylcholine receptor ligand binding is low in human brain and binding shows high inter-individual variation, we decided to measure mRNA expression and methylation in three regions of the promoter in human brain biopsies. For all three regions we find very low degree methylation. Interestingly, for the region surrounding the promoter we find a significant correlation between transcription and methylation. It is known that nicotine itself does not induce transcriptional upregulation of the α7 nicotinic acetylcholine receptor but a strikingly high proportion of schizophrenics are smokers and also users of antipsychotics known to modify epigenetic mechanisms. Therefore, we investigated if combinations of the two resulted in transcriptional upregulation and promoter demethylation. In SH-SY5Y cells we measured low degree methylation similar to that in human brain biopsies. Interestingly, transcription was not affected by valproate or nicotine alone whereas a combination of both resulted in promoter demethylation and transcriptional up-regulation. This suggests that some types of antipsychotics may potentiate the cognitive enhancing effects of nicotine in smoking patients.

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Poster

651. Anatomical Techniques: Optical Tissue Clearing

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Hope for Depression Research Foundation

Pritzker Neuropsychiatric Research Consortium

Title: Characterization of morphological changes in cleared tissue volumes using fluorescent microbead grid analysis.

Authors: *B. MARTIN, D. M. KROLEWSKI, V. KUMAR, H. AKIL, S. J. WATSON, Jr; MBNI, Univ. of Michigan, Ann Arbor, MI

Abstract: Recent advancements in tissue clearing methods and imaging techniques have provided researchers with the ability to analyze and measure intact three-dimensional structures within large volumes of brain tissue, without the use of physical sectioning of the tissue. While various clearing methods have been used successfully for this purpose, details about the expansion and contraction of the tissue caused by the clearing process are still unknown. In order to make accurate measurements and perform stereological analysis in cleared tissue samples, information is needed about overall change in tissue size. Four methods of tissue clearing (CLARITY, CUBIC, SeeDB, and 3Disco) were compared to measure changes in the size of the samples. To accomplish this, fluorescent microbeads were injected in a grid layout (100 μm gridline spacing) within pre-cleared tissue samples using a digital stereotaxic setup. Clearing was then performed on these tissue samples, and image volumes were acquired using a lightsheet microscope (CLARITY-Optimized Lightsheet Microscope). These volumes were visualized in AMIRA software and the resulting grids were measured. The results of this study will elucidate the details necessary to perform stereological analysis and measurements in tissue samples cleared by various methods.

Disclosures: B. Martin: None. **D.M. Krolewski:** None. **V. Kumar:** None. **H. Akil:** None. **S.J. Watson:** None.

Poster

651. Anatomical Techniques: Optical Tissue Clearing

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 651.02/NNN38

Topic: I.03. Anatomical Methods

Support: NIH:R01MH104261

ONR N00014-12-1-0366

Hope for Depression Research Foundation

Pritzker Neuropsychiatric Research Consortium

Title: Immunohistochemistry in fresh-frozen post-fixed brain tissue using antigen retrieval techniques

Authors: *D. M. KROLEWSKI, R. A. ILAGAN, V. KUMAR, A. PARSEGIAN, B. MARTIN, H. AKIL, S. J. WATSON, Jr;
Mol. and Behavioral Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI

Abstract: Background: Cataloging fresh-frozen brain tissue in the absence of prior aldehyde fixation and cryoprotection is beneficial for preserving protein and nucleic acids (RNA/DNA) across a variety of neuroscience methods requiring long-term storage. Unfortunately, detecting brain-localized antigens via immunohistochemistry (IHC) can be more challenging, if not impossible, using post-fixation following cryosectioning and slide-mounting. The inherent effect of autolysis, particularly during the thawing process, likely contributes to such marked loss of antigenicity (Jones et al., 1992). Slowly thawing frozen human brain blocks immersed in at subfreezing aldehyde-based fixative containing cryoprotectants can retain some degree of antigenicity otherwise lost under less ideal conditions (Jones et al., 1992). However, this technique also can require long incubation times in fixative/cyroprotectant for which thorough tissue penetration can be difficult to determine.**Methods and Results:** The present study evaluates more timely IHC methods in an effort to increase antigenicity using fresh-frozen rodent and human brain tissue. The effects of aldehyde-based and non-aldehyde precipitating fixatives were compared across an array of antibodies directed at methylation markers, glial cells, neuropeptides, and other neuronal-specific molecules on slide-mounted sections. In addition, each fixative was tested alone or paired with treatment of heated antigen-retrieval buffers at varied pH. Utilizing standard diaminobenzidine/horseradish peroxidase IHC amplification methods, we show that several antibodies including those specific for glia and particular subcortical neuronal populations are clearly expressed in well-characterized topographical patterns with relatively low background. Given the successful application in thin

slices, we have begun conducting similar experiments in 1 to 2mm-thick rodent and human brain slices using transparency techniques. 3-dimensional immunofluorescence in frozen tissue processed by CLARITY (Tomer et al., 2015), CUBIC (Susaki et. al., 2014), and SWITCH methods (Murray et. al., 2015) is being performed using both confocal and CLARITY Optimized Light Sheet Microscopy (COLM) systems. The results of this study will offer additional avenues for the processing of long-term stored fresh-frozen tissue.

Disclosures: D.M. Krolewski: None. **R.A. Ilagan:** None. **V. Kumar:** None. **A. Parsegian:** None. **B. Martin:** None. **H. Akil:** None. **S.J. Watson:** None.

Poster

651. Anatomical Techniques: Optical Tissue Clearing

Location: Halls B-H

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Program#/Poster#: 651.03/NNN39

Topic: I.03. Anatomical Methods

Support: NIH: R01MH104261

ONR N00014-12-1-0366

Hope for Depression Research Foundation

Pritzker Neuropsychiatric Research Consortium

Title: Optimization of the tyramide signal amplification based fluorescence *In situ* hybridization in cleared thick brain tissues

Authors: *V. KUMAR, D. M. KROLEWSKI, B. MARTIN, A. PARSEGIAN, H. AKIL, S. WATSON, Jr;
Mol. and Behavioral Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI

Abstract: Tyramide signal amplification provides extraordinary detection sensitivity to fluorescence *in situ* hybridization (FISH) to visualize targets with low expression. It is based on catalyzed reporter deposition (CARD) technique where peroxidase mediated formation of highly reactive tyramide radicals result in the binding of fluorochrome-conjugated tyramide only at the site of the enzymatic reaction. This method has been successfully used for thin section FISH studies over the years, however it has been still a challenge to utilize this system with recently developed CLARITY and other tissue clearing methods for high resolution imaging and molecular characterization within intact large volumes. One of the major challenges of tyramide-based amplification in thick tissues (100 μm and more) is the rapid deposition of most of the

available tyramide on the surface, exhausting the tyramide levels for deep layers of the tissue. Recent publications show a maximum signal penetration up to 100 µm depth using the commercially available tyramide kits. We explored this issue to achieve a uniform tyramide deposition across the tissue depth by using a combination of hydrolyzed cRNA probes and homemade biotin conjugated tyramide. Homemade biotinylated TSA is not only cost effective for larger tissue volumes but also provides an opportunity to regulate the catalysis/deposition of the tyramide. We characterized this improved method in fresh frozen rat- and CLARITY perfused mice-brain sections of various thickness. We are also comparing the mRNA detection in paraformaldehyde only fixed tissues (300 µm and 600 µm) and CLARITY tissues (1 mm) with or without 1-ethyl-3-3-dimethylaminopropyl carbodiimide (EDC) fixation. We are further exploring the multi-target detection with and without TSA and were able to visualize good signal strength by using a simple immunohistochemical detection of biotin labeled probes for some of the abundantly expressed mRNAs.

Disclosures: V. Kumar: None. **D.M. Krolewski:** None. **B. Martin:** None. **A. Parsegian:** None. **H. Akil:** None. **S. Watson:** None.

Poster

651. Anatomical Techniques: Optical Tissue Clearing

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 651.04/DP10 (Dynamic Poster)

Topic: I.03. Anatomical Methods

Title: A simple, improved tissue clearing method with broad adaptability for high fidelity molecular interrogation in intact whole brain

Authors: *Z. WU[1], R. AZEVEDO[1], R. FARRELL[1,2], N. RENIER[1], O. OLSEN[1], M. TESSIER-LAVIGNE[1];
[1]Lab. of Brain Develop. and Repair, Rockefeller Univ., New York, NY; [2]Dept. of Biochem., Weill Cornell Med., New York, NY

Abstract: The growing interest in understanding the complexity of organ-wide cellular interactions has led researchers to develop tissue clearing protocols for visualizing molecular patterns on a more global scale. Our lab recently developed iDISCO (Renier et al., 2014) for whole-mount immunolabeling in large cleared samples followed by volume imaging. iDISCO provides advantages for profiling distinct neuronal and non-neuronal cell populations, tracing long-range axonal projections, and examining physiological status (by using molecular reporters for neuronal activity, neural proliferation and degeneration) (Richardson and Lichtman, 2015). This enables efficient investigation of complex questions that were previously difficult to address

due to technical limitations.
In order to meet more versatile and challenging demands, we have systematically engineered and optimized each step of the clearing process. A first set of improvements led to the development of iDISCO+ (Renier et al., 2016), which provides more consistent labeling and clearing, and better preserves tissue morphology for automated data registration and analysis. We have built further on those advances to: 1) improve lipid removal to achieve isotropic optical clearing, while preserving native protein conformation. This would allow us to faithfully collect fluorescence protein signal or immunolabeling signal; 2) accelerate and improve molecular labeling; 3) further preserve sample size and fine tissue morphology after clearing; and 4) be compatible with both organic and aqueous imaging systems to enable broad microscopy applications. These improvements enable easy and reliable translation of traditional histological assays from sections to whole-mount tissues while maintaining or even improving sensitivity and coverage. The new method provides a powerful platform for investigating development, plasticity and degeneration in the intact nervous system.
Renier, N., Wu, Z., Simon, D.J., Yang, J., Ariel, P., and Tessier-Lavigne, M. (2014). iDISCO: a simple, rapid method to immunolabel large tissue samples for volume imaging. Cell *159*, 896-910.
Renier, N., Adams, E.L., Kirst, C., Wu, Z., Azevedo, R., Kohl, J., Autry, A.E., Kadiri, L., Venkataraju, K.U., Zhou, Y., Wang, V.X., Tang, C.Y., Olsen, O., Dulac, C., Osten, P., and Tessier-Lavigne, M. (2016). Mapping of brain activity by automated volume analysis of immediate early genes. Cell. DOI: 10.1016/j.cell.2016.05.007.
Richardson, D.S., and Lichtman, J.W. (2015). Clarifying Tissue Clearing. Cell *162*, 246-257.

Disclosures: Z. Wu: None. **R. Azevedo:** None. **R. Farrell:** None. **N. Renier:** None. **O. Olsen:** None. **M. Tessier-Lavigne:** None.

Poster

651. Anatomical Techniques: Optical Tissue Clearing

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 651.05/NNN40

Topic: I.03. Anatomical Methods

Support: ERC grant

Title: Microscopic imaging of optically cleared brain samples: two-photon laser scanning microscopy vs. confocal laser scanning microscopy

Authors: *A. SCHUETH[1], S. HILDEBRAND[2], R. GALUSKE[4], M. VAN ZANDVOORT[3], A. ROEBROECK[2];

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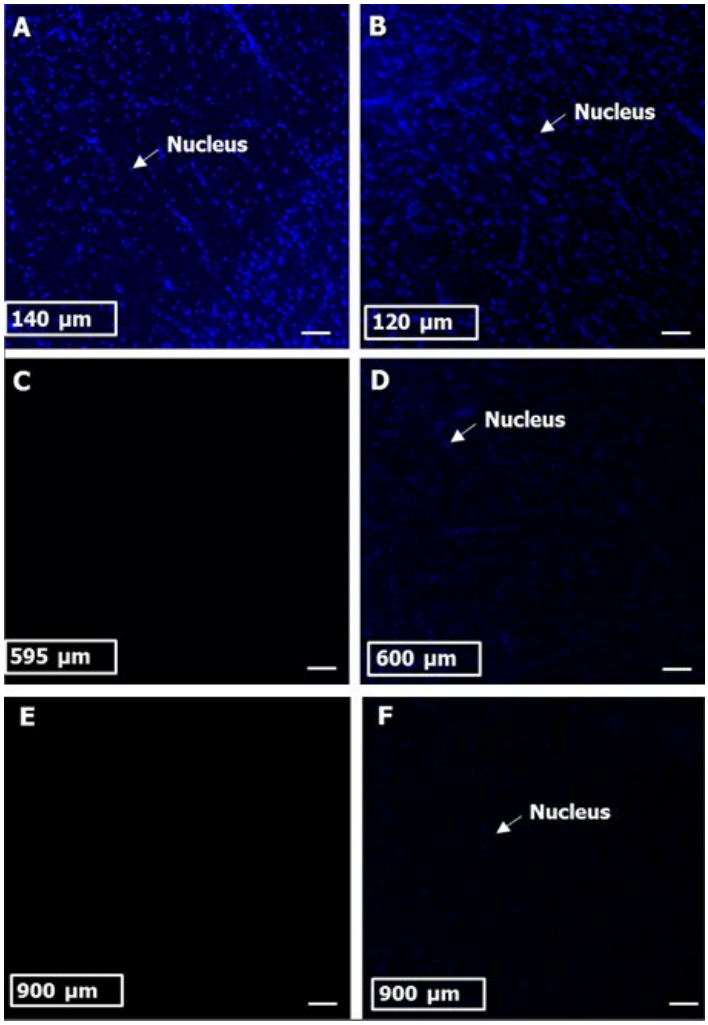
Abstract: Introduction
Our aim is a microscopic investigation of fixed, optically cleared brain tissue to visualize gray matter cytoarchitecture. To achieve this we performed comparative experimental imaging using confocal laser scanning microscopy (CLSM), as well as two-photon laser scanning microscopy (TPLSM) [1].
Methods
Formaline fixed, 3 mm thick pig brain samples were used. The clearing technique was a modified version of the FRUIT protocol (see Hildebrand et al., this conference [2]). DAPI (Carl Roth GmbH, Germany) was used for nuclear labelling. For imaging experiments a CLSM (Zeiss, AxioObserver LSM 780) was used, with a Plan-APO 10x/0.3 M27 objective. Excitation wavelength was 405 nm for DAPI. Laser power was 5-65 %. The field-of-view was 850.19 x 850.19 µm and the frame rate was 0.032 frames/sec. The TPLSM, a Leica TCS SP5 MP (Leica Mikrosysteme Vertrieb GmbH, Germany), had a HXC APO L 20x / 1.00 W 2mm working distance water immersion objective. Excitation wavelength was 800 nm and laser power 6-45%. During the examination the field-of-view was 738.10 x 738.10 µm and the frame rate 0.781 frames/sec.
Results & Conclusion
CLSM and TPLSM allowed the investigation of fixed, optical cleared brain tissue. With CLSM, cell nuclei (DAPI stained) were detected up to an imaging depth of approx. 600 µm within the sample (Fig 1c). TPLSM enabled the visualization of up to an imaging depth of more than 1 mm (Fig 1f) with showing stained nuclei (DAPI) throughout the complete sample thickness. We could show the advantage of TPLSM for neuroanatomical examinations of cleared brain samples for fluorescent labels with short excitation wavelengths. Future experiments with different optical clearing approaches could provide good imaging at depths of several millimeters.
Figures
Fig. 1: Brain samples were imaged with CLSM (A, C, E) and TPLSM (B, D, F). Imaging depths of the images are indicated in inserts. DAPI staining (nuclei, arrows) was visible up to an imaging depth of 600 µm using the CLSM (D) and up to approx. 900 µm with the TPLSM (F). Scale bars: 30 µm
References
1) Denk et al, 1990
2) Hildebrand et al, this conference



Disclosures: **A. Schueth:** A. Employment/Salary (full or part-time): Employment/Salary. **S. Hildebrand:** A. Employment/Salary (full or part-time): Employment/Salary. **R. Galuske:** None. **M. van Zandvoort:** None. **A. Roebroeck:** 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; ERC grant.

Poster

651. Anatomical Techniques: Optical Tissue Clearing

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 651.06/NNN41

Topic: I.03. Anatomical Methods

Support: ERC Grant

VIDI Grant

Title: Comparison of fructose/urea immersion-based protocols for optical clearing of brain tissue

Authors: *S. HILDEBRAND[1], K. VON WANGENHEIM[2], A. ROEBROECK[1], R. A. W. GALUSKE[2];
[1]Maastricht Univ., Maastricht, Netherlands; [2]Technische Univ. Darmstadt, Darmstadt, Germany

Abstract: Introduction
Recently optical clearing for 3D light microscopy of brain tissue is gaining attention in neuroscience. Some protocols that show a high clearing capacity remove the lipid components, making them incompatible with lipophilic dyes like DiI [1]. Approaches that leave lipid structures intact often show only limited clearing [2]. Here we compare clearing solutions based on the FRUIT protocol [3] for their ability to clear unperfused pig brain sections while still providing compatibility with DiI.
Methods
Three pig brain hemispheres were immersion fixed in 4 % PFA. Blocked hemispheres were cut into 3 mm coronal sections on a Leica VT 1000 S vibratome. For DiI staining, dye crystals were inserted into sulci of the slices, which were incubated at 37℃ for 4 months. DAPI staining was performed by incubating each slice in 6 ml of 1 µg/ml DAPI (CarlRoth) solution for 3 d at 4℃. For clearing, stepwise (10 %, 20 %, 40 %, 60 %, 80 %, 100 %) immersion in 20 ml each was performed for all three protocols. For each 100 % solution composition was as follows (percentages given in w/v respectively): FRUIT: 0.5 % thioglycerol, 11 % urea, 100 % fructose; Thiolgycerol: 100 % thioglycerol; Variant 4: 20 % thioglycerol, 48 % urea, 38.46 % fructose, 37 % sucrose, 3 % trehalose. Samples were incubated for 1 d (10 - 40 %), 2 d (60 %) and 3 d (80 - 100 %) at RT respectively. Imaging of the samples was performed on a Zeiss AxioObserver LSM 780.
Results & Conclusions
The Thioglycerol protocol led to tissue degradation and degraded stainings. Adding non-reducing sugars while increasing thioglycerol content up to 20 % in Variant 4, reduced the Maillard reaction and provided a high refractive index of 1.497. Clearing was superior to FRUIT and enabled deeper imaging of the DiI label. Filaments were visible to a depth of at least 1.5 mm and Somata for over 1 mm (Fig. 1a-d). No difference was seen for DAPI. We conclude that our FRUIT variant allows clearing samples with less Maillard reaction while leaving DiI labeling intact.
References
[1] Susaki, E. A., et al. (2015)
[2] Ke, M. T. and T. Imai (2014)
[3] Hou, B., et al. (2015)

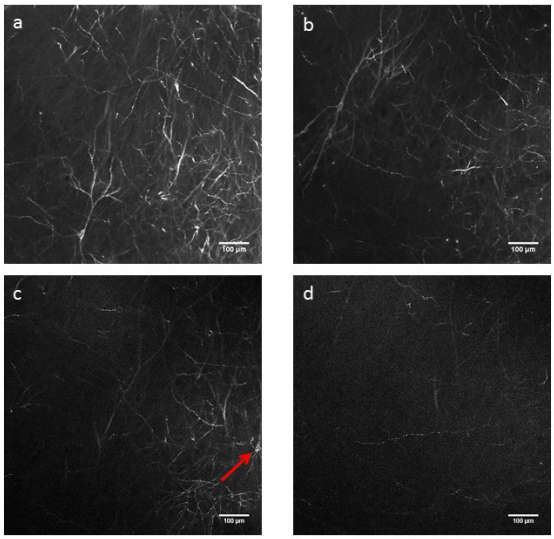


Figure 1: Example of DiI stained pig brain sample, cleared with Variant 4. Images were acquired with Confocal Laser Scanning Microscopy (CLSM) at depths of 525 µm (a), 805 µm (b), 1155 µm (c) and 1505 µm (d) respectively. Cell bodies are visible to at least 1000 µm (a, b, c: arrow). Labeled filaments could be detected to a depth of at least 1500 µm (d).

Disclosures: S. Hildebrand: None. **K. von Wangenheim:** None. **A. Roebroeck:** None. **R.A.W. Galuske:** None.

Poster

651. Anatomical Techniques: Optical Tissue Clearing

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 651.07/NNN42

Topic: I.03. Anatomical Methods

Support: KIST Joing Research Lab

Title: Combining CLARITY with mGRASP for brain-wide mapping of projections and synapses with light microscopy

Authors: *S.-Y. KIM[1], H.-E. PARK[1], D.-J. KOO[1], M. CHOE[1], H. LEE[2], J. KIM[2];
[1]Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: Complete mapping of neural connectivity across the mammalian brain (the "connectome") is a daunting and exciting prospect. Achieving this goal, even in its simplest form, requires the method that enables tracing and reconstruction of all neurites and their branches while determining the exact location and type of synapses. With relevance for this goal,

various clearing and labeling techniques for large-scale tissues are emerging, including CLARITY, stochastic electrotransport and SWITCH (Chung *et al., Nature* 2013, Kim *et al., PNAS* 2015, Murray *et al., Cell* 2015). These techniques render the tissues transparent and enable rapid and multi-round labeling, which may allow for light microscope-based imaging of all neural cell bodies, their dendrites and short- and long-range projections across the entire brain. However, the cutting-edge light microscopes optimized for the large-scale samples cannot accurately detect nanometer-scale synapses, due to the diffraction limitations of light microscopy. Therefore, the tissue clearing and labeling techniques alone do not easily lend support to synapse-by-synapse mapping of the neural circuitry. To overcome this, we aim to combine the advanced form of tissue clearing and labeling techniques with mGRASP (mammalian GFP reconstruction across synaptic partners) (Kim *et al., Nat Methods* 2012). mGRASP is the technique that enables reliable detection of mammalian synapses with light microscopy, taking advantage of the complementation of split GFP partners across the synapse. Our goal is to develop a reliable tool for the detailed mapping of neural connectivity over the millimeter-range.

Disclosures: S. Kim: None. **H. Park:** None. **D. Koo:** None. **M. Choe:** None. **H. Lee:** None. **J. Kim:** None.

Poster

651. Anatomical Techniques: Optical Tissue Clearing

Location: Halls B-H

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Program#/Poster#: 651.08/NNN43

Topic: I.03. Anatomical Methods

Support: Vascular Dementia Research Foundation

Synergy Excellence Cluster Munich (SyNergy)

ERA-Net Neuron (01EW1501A to A.E.)

Graduate School of Neuroscience (GSN) Ludwig Maximilian University of Munich

Title: Visualization of adult rodent bodies at the sub-cellular level using uDISCO tissue clearing

Authors: C. PAN, R. CAI, F. P. QUACQUARELLI, A. GHASEMI, *A. ERTURK;
Inst. for Stroke and Dementia, Munich, Germany

Abstract: Optical tissue clearing approaches started to revolutionize standard histology by allowing to image intact organs and organisms. Organic solvents used in 3DISCO[1,2] including

BABB or DBE known to achieve the highest level of transparency among all reported clearing approaches. Hence, already in last 1-2 years 3DISCO has been used to study immune cells[3], stem cells[4,5], cancer cells[6,7] and transdifferentiating lung cells[8]. It has also been combined with deep tissue antibody labeling methods[9,10]. However, because 3DISCO quenches the signal from endogenous fluorescent proteins fast (half life of a few days)[1], it was not applicable to large samples requiring longer clearing procedure such as adult rodent bodies. Here, we developed ultimate (u)DISCO clearing approach, which preserves the endogenous fluorescence signal 8-10 times better compared to 3DISCO. uDISCO uses a novel tissue clearing method, which renders intact organs and rodent bodies transparent while reducing their size up to 65%, enabling standard light-sheet microscopy imaging on whole adult rodent bodies without physical sectioning. We used uDISCO to image neuronal connections and vasculature from head to toe over 7-10 centimeters and trace transplanted stem cells in an unbiased way throughout the entire bodies of adult mice. uDISCO is compatible with various labeling methods including virus tracing, antibody labeling and it is also applicable to over-fixed human tissues. Thus, uDISCO is a robust tissue-clearing method applicable to diverse biomedical purposes. 1. Erturk, A. *et al. Nat Protoc* **7**, 1983-1995, (2012). 2. Erturk, A. *et al. Nat Med* **18**, 166-171, (2012). 3. Liu, Z. *et al. Nature* **528**, 225-230, (2015). 4. Acar, M. *et al. Nature* **526**, 126-130, (2015). 5. Espinosa-Medina, I. *et al. Science* **345**, 87-90, (2014). 6. Oshimori, N. *et al. Cell* **160**, 963-976, (2015). 7. Garofalo, S. *et al. Nature communications* **6**, 6623, (2015). 8. Lafkas, D. *et al. Nature* **528**, 127-131, (2015). 9. Renier, N. *et al. Cell* **159**, 896-910, (2014). 10. Belle, M. *et al. Cell reports* **9**, 1191-1201, (2014).

Disclosures: **C. Pan:** None. **R. Cai:** None. **F.P. Quacquarelli:** None. **A. Ghasemi:** None. **A. Erturk:** None.

Poster

651. Anatomical Techniques: Optical Tissue Clearing

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Program#/Poster#: 651.09/NNN44

Topic: I.03. Anatomical Methods

Support: Seed Funding Program for Applied Research 201409160006

Title: Development of tissue clearing techniques for the visualisation of neurodegenerative pathology in three-dimensions

Authors: ***R. C. CHANG**[1,2,3], A. K. L. LIU[1,4], H.-M. LAI[5], M. E. D. HURRY[6], J. DEFELICE[4], S. M. GENTLEMAN[4];
[1]Lab. of Neurodegenerative Diseases, LKS Fac. of Medicine, Univ. of Hong Kong, Hong Kong,

China; [2]Res. Ctr. of Heart, Brain, Hormone and Healthy Aging, LKS Fac. of Medicine, The Univ. of Hong Kong, Hong Kong, China; [3]State Key Lab. of Brain and Cognitive Sciences, The Univ. of Hong Kong, Hong Kong, China; [4]Neuropathology Unit, Div. of Brain Sciences, Dept. of Medicine, Imperial Col. London, London, United Kingdom; [5]Sch. of Biomed. Sciences, LKS Fac. of Medicine, The Univ. of Hong Kong, Hong Kong, China; [6]Nuffield Dept. of Clin. Neurosciences, Univ. of Oxford, Oxford, United Kingdom

Abstract: Introduction: Recent advancement of laser microscopy and endogenous fluorescent tagging techniques has led to the development of many tissue clearing strategies, which render tissues optically transparent, allowing large blocks of unsectioned tissue to be visualised in 3D. Although there have been a few successful attempts at clearing of human brain tissue, challenges remain in achieving immunolabelling to a significant depth of the tissue, as well as clearing of large tissue blocks in densely myelinated brain regions. Therefore, we aim to develop an improved and simplified tissue clearing protocol for 3D visualisation of neurodegenerative pathology in the human brain. **Methods:** Fresh and formalin-fixed tissue from various regions in the human brain were processed and immunostained using CLARITY, 3DISCO and/or iDISCO. CLARITY-treated samples were immersed in different refractive index matching media before mounting and visualising under a confocal microscope. **Results:** We have optimised the CLARITY protocol for use on human tissue for the visualisation of Lewy pathology in 3D. Furthermore, by combination of different techniques, we have developed a novel tissue clearing protocol for human brain tissues, which improves immunolabeling and reduces the overall processing time from tissue fixation to immunostaining and visualisation to a minimum of 16 days. **Conclusions:** A greatly simplified and more user-friendly tissue clearing protocol has been developed for the 3D visualisations of human brain tissue. However, further work is needed for the improvement in immunolabeling and clearing of archival formalin-fixed tissue materials.

Disclosures: R.C. Chang: 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; Seed Funding Program for Applied Research 201409160006. **A.K.L. Liu:** None. **H. Lai:** None. **M.E.D. Hurry:** None. **J. DeFelice:** None. **S.M. Gentleman:** None.

Poster

651. Anatomical Techniques: Optical Tissue Clearing

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Program#/Poster#: 651.10/NNN45

Topic: I.03. Anatomical Methods

Support: NFR-2012M3A9C6049933

NRF-2015M3C7A1028790

KBRI-2231-415

NRF-2015M3C7A1030964

NRF-2015M3C7A1028396

Title: ACT-PRESTO: Rapid and consistent tissue clearing and labeling method for 3-dimensional (3D) imaging

Authors: ***E. LEE**[1], J. CHOI[1], Y. JO[1], J. KIM[1], Y. JANG[2], H. LEE[2], S. KIM[3], H.-J. LEE[4], K. CHO[4], N. JUNG[4], E. HUR[5,6], I. RHYU[1], H. KIM[1], S. JEONG[2], Y. CHOE[2], C. MOON[3], W. SUN[1];
[1]Korea Univ. Col. of Med., Seoul, Korea, Republic of; [2]Korea Brain Res. Inst., Daegu, Korea, Republic of; [3]Daegu Gyeungbuk Inst. of Sci. and Technol. (DGIST), Daegu, Korea, Republic of; [4]Logos Biosystems, Inc., Gyunggi-Do, Korea, Republic of; [5]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [6]Korea Univ. of Sci. and Technol. (UST), Daejeon, Korea, Republic of

Abstract: Imaging cellular and subcellular structures in an intact organ or organism is a fundamental challenge in biology. Conventionally, this task has been approached by reconstructing immuno-labeled images taken from a series of tissue sections into a 3-dimensional structure. However, this approach is labor- and resource-intensive. A more critical limitation of this approach is that serial-image reconstructing is not accurate because physical sectioning distorts tissues and registration of two consecutive images is prone to errors. The recent advances of tissue clearing techniques allow visualization of cellular structures and neural networks inside of unsectioned whole tissue or even whole body. However, currently available protocols require long process time. Here, we present a rapid and highly reproducible ACT-PRESTO (active clarity technique-pressure related efficient and stable transfer of macromolecules into organs) method that renders tissue or whole-body clearing within a day while preserving tissue architecture and protein-based signals derived from endogenous fluorescent proteins. Moreover, ACT-PRESTO is compatible with conventional immunolabeling methods and expedites antibody penetration into thick specimens by applying pressure. Speed and consistency of this method will allow high-content mapping and analysis of normal and pathological features in intact organs and bodies.

Disclosures: **E. Lee:** None. **J. Choi:** None. **Y. Jo:** None. **J. Kim:** None. **Y. Jang:** None. **H. Lee:** None. **S. Kim:** None. **H. Lee:** None. **K. Cho:** None. **N. Jung:** None. **E. Hur:** None. **I. Rhyu:** None. **H. Kim:** None. **S. Jeong:** None. **Y. Choe:** None. **C. Moon:** None. **W. Sun:** None.

Poster

651. Anatomical Techniques: Optical Tissue Clearing

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 651.11/NNN46

Topic: I.03. Anatomical Methods

Support: Dan and Martina Lewis Biophotonics Fellowship WABC-DMLF-20141106

Title: Quantitative assessment of 3D imaging for blood vessel patterns in the CNS

Authors: *J. OGAWA, G. M. PAO, I. M. VARMA;
Salk Inst. LOG-V, La Jolla, CA

Abstract: Here we describe 3D imaging of blood vessel patterning in the mouse CNS and investigate the quantitative properties of their branching pattern at different scales. A large variety of techniques have been tried for visualization of vascular structure, but with section staining, it was difficult to obtain the whole context of complicated structures. We apply a new technique to make tissue transparent which allows us to image the complicated blood vessels patterns in the CNS without losing information from sectioning. With these techniques and using confocal laser-scanning microscopy, we succeeded to image the brain to approximately 600 um in depth. The image is of enough contrast for quantitative assessment of the detailed structure of small blood vessels. We used these 3D image datasets to investigate the quantitative properties of brain blood vessels at different scales. This new assessment shows significant differences in the blood vessel patterning in different areas of the brain. Changes in total Cerebral blood flow with aging or neural stimulus has been observed using PET or MRI imaging. We will use these newly established assessment to investigate whether aging related or neural stimulus related brain state changes also induce blood vessel state differences in different areas of the brain.

Disclosures: J. Ogawa: None. **G.M. Pao:** None. **I.M. Varma:** None.

Poster

651. Anatomical Techniques: Optical Tissue Clearing

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 651.12/NNN47

Topic: I.03. Anatomical Methods

Support: NIH / NINDS (NS085770)

NIH / NIA (AG013854)

Title: Three-dimensional visualization of cellular and disease markers in human brain using tissue clearing methods

Authors: Z. PARTON, L. KUKREJA, T. GEFEN, A. REZVANIAN, E. H. BIGIO, M.-M. MESULAM, *C. GEULA;
Cogn Neurol & Alzhei Dis Cent, Northwestern Univ. Med. Sch., Chicago, IL

Abstract: Recent advances in methods for optical tissue clearing have enabled 3D visualization of long-range neuronal circuitry and detailed cellular structures. These procedures remove lipid bilayers while preserving cellular proteins to ultimately render tissue transparent such that it can be fluorescently labeled and imaged. To date, few studies have applied these methods to human specimens. The Cognitive Neurology and Alzheimer's Disease Center (CNADC) at Northwestern University seeks to optimize clearing protocols for application to postmortem human tissue with the aim of conducting large scale 3D analysis of anatomic and pathologic features of neurodegenerative diseases. We have successfully applied the "CLARITY" and "iDISCO" protocols to blocks of human motor and cerebellar cortices. Each method carries relative benefits and disadvantages, and our trials have enabled us to fine-tune these procedures for relatively large human sections. CLARITY, also known as **C**lear **L**ipid-exchanged **A**crylamide-hybridized **R**igid **I**maging-compatible **T**issue-h**Y**drogel is an aqueous-based clearing method that utilizes hydrogel embedding, detergent, and/or electrophoresis followed by antibody labeling to produce the desired effects. In contrast, the iDISCO method (**I**mmunolabeling-enabled three-**D**imensional **I**maging of **S**olvent-**C**leared **O**rgans) starts with antibody labeling, followed by incubation of the tissue in a series of organic solvents to dehydrate and delipidate its content. Both methods produced 'cleared' (i.e. transparent) cortical and cerebellar tissue, with a high signal-to-noise ratio when imaged with a laser scanning confocal and multi-photon microscopes. When visualizing structures in 3D, we were able to render high resolution z-stacks that exceeded depths of 1mm. We also extended the application of our methods to brains with neurodegenerative disorders. Our immunolabeling method visualized neuronal, glial, and pathologic markers, including amyloid plaques, neurofibrillary tangles and TDP-43 inclusions. Overall, we present here methodologies for clearing human tissue that are invaluable to the study of neurodegenerative diseases. This will allow us to observe fine-scale anatomic distribution of pathology, and significantly inform systems-level understanding of intact and impaired cognitive functions.

Disclosures: Z. Parton: None. **L. Kukreja:** None. **T. Gefen:** None. **A. Rezvanian:** None. **E.H. Bigio:** None. **M. Mesulam:** None. **C. Geula:** None.

Poster

651. Anatomical Techniques: Optical Tissue Clearing

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 651.13/NNN48

Topic: I.03. Anatomical Methods

Support: NYSTEM C029157

Title: Comparison and optimization of tissue clearing techniques for whole-brain imaging of indelibly labeled memory traces

Authors: *I. PAVLOVA, S. C. SHIPLEY, R. HEN, C. A. DENNY;
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Abstract: Whole-brain tissue clearing and microscopy imaging is a rapidly growing area of focus. We evaluated several tissue clearing and labeling approaches, such as iDISCO, CLARITY, PACT, and CUBIC, to label and visualize whole-brain memory traces in intact brains from the ArcCreER^{T2} mouse model. This model labels neural ensembles activated during memory encoding by expression of channelrhodopsin (ChR2)-enhanced yellow fluorescent protein (eYFP) and neural ensembles activated during memory retrieval by immunolabeling of Arc and/or c-Fos. Our goal was to develop a technique that allows for homogenous whole-brain clearing, immunolabeling, and imaging of both the densely expressed eYFP label and the sparser Arc/c-fos label. We first evaluated several protocols and found that the best results were achieved by iDISCO rather than CLARITY or PACT. However, using the original iDISCO protocol, uniform thick-tissue immunolabeling was achieved only for Arc/c-fos, but not for the densely expressed eYFP label. Next, we modified the original iDISCO protocol for compatibility with a GFP nanobody, a functionally similar but physically smaller alternative to a GFP IgG antibody. We found that the nanobody penetrated deep tissue better than its whole antibody counterpart, making it a potential candidate for improved eYFP iDISCO-based immunolabeling. Finally, we evaluated several clearing approaches for preservation of endogenous eYFP fluorescence and co-staining with Arc and/or c-fos. CUBIC (with reagent 1A) showed the best preservation of endogenous eYFP fluorescence, with compatibility for Arc immunolabeling in thick tissue sections. These results suggest iDISCO and/or CUBIC as the whole-brain clearing methods of choice for compatibility with the ArcCreER^{T2} mouse line. The use of these methods will provide a novel approach to studying memory traces across the entire brain and new insights into the circuits that underlie memory, cognition, and neuropsychiatric disorders.

Disclosures: I. Pavlova: None. **S.C. Shipley:** None. **R. Hen:** None. **C.A. Denny:** None.

Poster

652. New Assays for Monitoring Cells, Circuits, and Behavior

Location: Halls B-H

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Program#/Poster#: 652.01/NNN49

Topic: I.04. Physiological Methods

Support: FWO grant

Title: Longitudinal cellular imaging of the astrocyte response to chronically implanted neural probes

Authors: *K. MOLS[1,2,3], S. MUSA[1], B. NUTTIN[2], L. LAGAE[1,2], V. BONIN[3];
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Abstract: Neural implants for electrical recordings or stimulation are critical for studying neural circuits, for building brain computer interfaces, and for restoring brain function. Minimizing the tissue damage and foreign body reaction is key to the success of such implants. Most knowledge about the device-tissue interface is obtained with end-point methods such as histology. However, the ability to monitor the progression of the foreign body response *in vivo* and to correlate it to implant performance would greatly contribute to our understanding of the dynamics of the device-tissue interaction.

To address this issue, we present an *in vivo* approach to (1) longitudinally image the cellular environment surrounding an implant, and (2) reliably record electrophysiological data from that implant. We used this tool to study the relation between the astrocytic reaction to a multisite single-shank silicon probe, and the stability of long-term neuronal recordings.

We combined a chronic cranial window over the visual cortex with a functional probe in a transgenic mouse, expressing the green fluorescent protein (GFP) under control of the astrocyte-specific Aldh1l1-promotor. Using a video-rate two-photon (2P) microscope, we imaged 500 μm² optical planes between 200 and 500 μm subdural weekly for 10 consecutive weeks. We corrected these images for variations in brain transparency and quantified GFP fluorescence around the probe as a measure for astrocyte reactivity. Before each imaging session, we recorded spiking activity under light ketamine anesthesia and calculated the average signal and noise amplitude in order to monitor the probe's performance. At the end of the experiment, we used confocal microscopy to image immunohistochemically labelled astrocytes (GFAP), microglia (IBA1) and nuclear DNA (DAPI) for each animal (N = 8).

We found that 2P imaging can resolve astrocytic processes at the probe's surface. The average fluorescence signal of both cell somas and processes did not show significant variation for 10 weeks (one-way univariate ANOVA, p = 1,0). Similarly, noise and spike amplitude remained constant. The noise (12 μV_{rms} on average) never reached the amplitude range of the spiking activity (123 μV_{pp} on average). Finally, the histological markers did not show a marked build-up

of inflammatory cells around the probe.
We show that our approach provides a unique look into the cellular changes at the probe-tissue interface and allows us to longitudinally monitor the foreign body response while the probe is implanted. Moreover, our results indicate that a stable astrocytic response to an implant coincides with stable electrical recordings.

Disclosures: **K. Mols:** None. **S. Musa:** None. **B. Nuttin:** None. **L. Lagae:** None. **V. Bonin:** None.

Poster

652. New Assays for Monitoring Cells, Circuits, and Behavior

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Topic: I.04. Physiological Methods

Support: Ontario Brain Institute

NeuroDevNet Canadian Network of Centres of Excellence

Halbert Chair in Neural Repair and Regeneration

DeZwirek Foundation

Title: A novel CNS white matter preparation for electrophysiological studies of myelinated and non-myelinated axons: the isolated corpus callosum from mouse brain

Authors: ***A. A. VELUMIAN**[1,4], L. LI[1], M. SAMOILOVA[1], M. G. FEHLINGS[1,5,2,3];
[1]Toronto Western Hosp. / Neurosurg., [2]Krembil Neurosci. Ctr., [3]Krembil Res. Inst., Univ. Hlth. Network, Toronto, ON, Canada; [4]Departments of Surgery and Physiol., [5]Dept. of Surgery, Univ. of Toronto, Toronto, ON, Canada

Abstract: The axonal function in CNS white matter of laboratory animals is studied by recording compound action potentials (CAPs). In the optic nerve or dissected spinal cord white matter, reliable CAP recordings are done similar to peripheral nerves by using suction electrodes, grease gap or sucrose gap techniques for stimulation and recording of large axonal populations. The corpus callosum (CC), a major white matter structure interconnecting brain hemispheres, is extensively used for studying CNS axonal function. Unlike the optic nerve where all axons are myelinated, the CC contains also a large population of non-myelinated axons, making it particularly useful for studying both types of axons in various physiological and pathological conditions. CAP recordings in CC are typically done with extracellular microelectrodes that limit

the recordings to a limited axonal population around the electrode tip. Here we introduce a novel robust isolated "nerve-like" CC preparation that allows for suction electrode stimulation and recording similar to approaches used in the optic nerve. Unlike microelectrode CAP recordings in brain slices or in vivo, where the CC CAP peaks representing myelinated and non-myelinated axons vary broadly in size, the "nerve-like" CC CAPs show stable reproducible ratios of these two main peaks, and also reveal a third peak, suggesting a distinct group of smaller caliber non-myelinated axons. We will provide detailed characterization of "nerve-like" CC CAPs and conduction velocities of myelinated and non-myelinated axons within CC at different positions along the rostro-caudal axis of CC body. The "nerve-like" CC preparation has advantages for studying the changes in axonal conduction properties due to demyelination, which we will illustrate by comparing the CC CAPs in wild type and dysmyelinated *shiverer* mice. Similar to other nerve or nerve-like preparations, the isolated CC is useful for studying the effects of temperature, bath-applied drugs (4-aminopyridine) and ischemia (modeled by oxygen-glucose deprivation) on axonal function. Due to isolation from gray matter, the “nerve-like” CC preparation allows for studying the axonal function without "contamination" by reverberating signals from gray matter. Our analysis of "whole" CC CAPs in this nerve-like preparation reveals higher complexity of myelinated and non-myelinated axonal populations, not noticed earlier. This preparation may have a broad range of applications as a robust model for studying myelinated and non-myelinated axons of the CNS in various experimental models.

Disclosures: **A.A. Velumian:** None. **L. Li:** None. **M. Samoilova:** None. **M.G. Fehlings:** None.

Poster

652. New Assays for Monitoring Cells, Circuits, and Behavior

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Topic: I.04. Physiological Methods

Support: NIH Grant 1R21EB018494-01A1

Title: Physiological basis of high frequency resting state networks in the human brain using high-speed fMRI and computer simulations of spatial autocorrelations

Authors: C. TRAPP[1], K. VAKAMUDI[1], M. O. CHOHAN[2], *S. POSSE[1];
[1]Neurol., [2]Neurosurg., Univ. of New Mexico, Albuquerque, NM

Abstract: Current studies of resting-state connectivity rely on coherent signal fluctuations at frequencies around 0.1 Hz, however, recent studies have shown that fluctuations above 0.5 Hz may exist. This may permit the observation of functional connectivity dynamics at even shorter

time scales than is currently feasible at frequencies < 0.3 Hz. The current study replicates the feasibility of measuring high frequency (HF) correlations in six healthy controls and a patient with a brain tumor in left parietal lobe, while analyzing non-physiological sources via simulation.
Resting-state data were acquired using a high-speed multi-slab echo-volumar imaging (MEVI) pulse sequence, which increases BOLD sensitivity compared to EPI (TR: 136 ms, spatial resolution: $4\times4\times6$ mm^3). Bandpass filtering of low frequency connectivity and physiological noise, in combination with a confound-tolerant sliding-window seed-based connectivity analysis (WSCA) using running mean/standard deviation was used to map HF correlations. Whole Brodmann areas (BA) and single voxels derived from peak correlations were used as seeds. Simulations of Rician noise and the underlying point-spread function (PSF) were analyzed to estimate baseline auto-correlation levels in the four networks studied (auditory, sensorimotor, visual, and default-mode).
Only the auditory and default mode networks were observed to have HF correlations significantly above baseline auto-correlation levels when observed with BA seeds. When analyzed with peak voxel seeds, auditory was the only network to show extended or bilateral correlations. All other networks were restricted to PSFs. Additionally, HF auditory correlations in the tumor patient showed a spatial displacement anterior to the tumor, corresponding with a similar displacement observed at low frequencies. When seeded within the tumor itself, there were no HF correlations outside of the PSF, in contrast with a contralateral seed.
This study demonstrates resting-state connectivity at frequencies > 0.5 Hz in the auditory and default-mode networks using sensitivity enhanced MEVI acquisition, confound tolerant WSCA and simulations of baseline autocorrelations. The displacement of high frequency correlation maps in the tumor patient as a result of the lesion further supports the physiological nature of observed high frequency correlations. While this does not rule out the possibility of visual and sensorimotor networks at high frequencies, their existence cannot be determined with the current SNR and temporal resolution limits of this study. Further studies are required to assess the origin of these high frequency correlations.

Disclosures: **C. Trapp:** None. **K. Vakamudi:** None. **M.O. Chohan:** None. **S. Posse:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Founder of NeurInsight, a startup company to share the TurboFIRE fMRI analysis tool.

Poster

652. New Assays for Monitoring Cells, Circuits, and Behavior

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Topic: I.04. Physiological Methods

Support: Lundbeck Foundation

Fondation Jucum, Denmark

Simon Fougner Hartmans, Denmark

Title: Safety and EEG data quality of concurrent high-density EEG and high-speed fmri at 3 Tesla

Authors: *O. B. PAULSON[1], M. T. FOGED[1], U. LINDBERG[2], K. VAKAMUDI[3], H. B. W. LARSSON[1], L. PINBORG[1], T. W. KJÆR[4,5], M. FABRICIUS[2], C. SVARER[2], B. OZENNE[5], L. WU[3,6], V. D. CALHOUN[3,6], B. FISH[3], C. THOMSEN[1], S. BENICZKY[2,7,8], S. POSSE[3]; [1]Rigshospitalet & Univ. of Copenhagen, Copenhagen, Denmark; [2]Rigshospitalet, Copenhagen, Denmark; [3]Univ. of New Mexico, Alberqerque, NM; [4]Roskilde Hosp., Roskilde, Denmark; [5]Univ. of Copenhagen, Copenhagen, Denmark; [6]Mind Res. Network, Alberqerque, NM; [7]Aarhus Univ. Hosp., Aarhus, Denmark; [8]The Epilepsy Hosp., Dianalund, Denmark

Abstract: ***Aims:*** Concurrent EEG and fMRI is increasingly used to characterize the spatial-temporal dynamics of brain activity in neuroscience and clinical applications. However, most studies to date have been limited to using conventional echo-planar imaging. There is now increasing interest to integrate recently developed high-speed fMRI methods with high-density EEG arrays to increase temporal resolution and sensitivity for resting state fMRI and for detecting interictal spikes in patients with epilepsy. ***Methods:*** In this study we investigate radiofrequency related heating in concurrent high-density EEG and high-speed fMRI using three, clinical, 3 Tesla MR scanners from two different vendors equipped with a 64- and 256-channel MR-compatible EEG system comparing EPI, multi-echo EPI, MB-EPI and MS-EVI pulse sequences. In addition, EEG data quality was assessed on one of the scanners using a 64-channel EEG system. Data were collected in 10 healthy controls and 14 patients with epilepsy. ***Results:*** Radiofrequency heating at the surface of the EEG electrodes during 20 min scans did not exceed 1.1^{o} C. The EEG data quality during the MR scan was deteriorated compared to EEG outside of the scanner, primarily due to residual gradient artifacts and movement. The EEG quality was not significantly dependent on the fMRI method used, but a trend towards deterioration of the EEG quality with increasing short-term movement was found. ***Conclusion:*** This study demonstrates that high-density EEG can be safely implemented in conjunction with high-speed fMRI methods under the experimental conditions of this study, but deterioration of EEG quality inside the MR scanner may limit clinical applications.

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Poster

652. New Assays for Monitoring Cells, Circuits, and Behavior

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Topic: I.04. Physiological Methods

Support: NIH Grant NS069909

NIH Grant NS078680

NIH Grant NS093669

Title: Resting state fMRI signals in white matter of non-human primates

Authors: T.-L. WU, F. WANG, A. W. ANDERSON, L. CHEN, Z. DING, *J. C. GORE; Vanderbilt Univ., Nashville, TN

Abstract: Neither blood oxygenation level dependent (BOLD) activation nor resting state magnetic resonance imaging (rsfMRI) signals in white matter have been well-established. However, growing evidence suggests reliable detection of white matter BOLD signals may be possible. We have reported the measurement of anisotropic resting state correlations within white matter that reveal an underlying functional structure. Recently, we introduced the concept of a functional correlation tensor (FCT) as a description of the functional architecture of white matter, purely on the basis of rsfMRI data. We find these FCTs delineate long range white matter tracts as well as short range sub-cortical fibers imaged at rest, suggesting temporal resting state signals may reflect intrinsic synchronizations of neural activity in white matter. In order to evaluate the neural basis of these correlated fluctuations, we assessed whether MRI signal fluctuations in white matter vary for different baseline levels of neural activity. Specifically, we performed imaging studies on live squirrel monkeys under different levels of isoflurane anesthesia at 9.4T. Our results showed that the power in both gray and white matter low frequencies decreased monotonically in similar manner with increasing levels of anesthesia. Moreover, the distribution of fractional anisotropy values of the functional tensors in white matter were significantly higher than those in gray matter. In addition, the functional tensor eigenvalues (i.e. greater nearest neighbor resting state correlations) decreased with increasing level of anesthesia. Overall, our results suggest that as anesthesia level changes baseline neural activity, white matter signal fluctuations behave similarly to those in gray matter, and functional tensors in white matter are affected in parallel. These findings confirm that BOLD-like signal fluctuations in white matter can be detected in a resting state, are anisotropic in nature, and consistent with underlying neural activity.

Disclosures: **T. Wu:** None. **F. Wang:** None. **A.W. Anderson:** None. **L. Chen:** None. **Z. Ding:** None. **J.C. Gore:** None.

Poster

652. New Assays for Monitoring Cells, Circuits, and Behavior

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Topic: I.04. Physiological Methods

Support: ERC grant 339244 - FUSIMAGINE

ANR-10-LABX-24 LABEX WIFI

ERA-FLAG FUSIMICE

Title: Transcranial functional ultrasound imaging in freely-moving awake mice without contrast agent through the intact skull

Authors: J. FERRIER[1], E. TIRAN[2], T. DEFFIEUX[2], J.-L. GENNISSON[2], S. PEZET[1], M. TANTER[2], *Z. LENKEI[3];
[1]CNRS UMR 4289, ESPCI-ParisTech, PSL Res. Univ., Paris, France; [2]Inst. Langevin, INSERM U979, ESPCI-ParisTech, Paris, France; [3]ESPCI-ParisTech, Paris, France

Abstract: Ultrasensitive ultrasound Doppler imaging enables fMRI-like dynamic measurement of cerebral blood volume with high-sensitivity and has recently led to the development of functional ultrasound (fUS) imaging. fUS imaging is a powerful tool for measuring brain activation and connectivity through the neurovascular coupling with high spatiotemporal sampling (100 µm, 1 ms), Here we investigated the use of fUS imaging of mice, an important pre-clinical model. First we show that ultrasensitive Doppler imaging of the entire brain is possible directly and completely non-invasively through the intact skull and skin without contrast agents in anesthetized mice, regardless of age. Using a motorized probe, we also performed non-invasive high-resolution 3D Doppler tomography in adult mice. Next, we demonstrate the feasibility of high-quality full-transcranial fUS imaging in awake and freely-moving mice in a minimally invasive setting. For this purpose we magnetically clipped a newly developed ultralight ultrasound probe to a small and flat metal frame that we fixed to the mouse skull. We have also developed a dedicated ultrasonic sequence to avoid a previously unreported specific muscle-related artifact in awake rodents. This setup allowed us to acquire full-depth high-resolution images of the awake mouse brain vasculature as well as detect the activation of the barrel cortex during whisker stimulation. These results open new perspectives for the use of fUS

imaging in imaging of behavioral studies and in non-invasive longitudinal studies of postnatal brain development.

Disclosures: **J. Ferrier:** None. **E. Tiran:** None. **T. Deffieux:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroFlows. **J. Gennisson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroFlows. **S. Pezet:** None. **M. Tanter:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroFlows. **Z. Lenkei:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroFlow.

Poster

652. New Assays for Monitoring Cells, Circuits, and Behavior

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Topic: I.04. Physiological Methods

Support: Friedrich Miescher Institute for Biomedical Research

Novartis Institutes for Biomedical Research

Title: Effects of autism-related genetic modifications in zebrafish on social behavior and neural activity

Authors: ***K.-H. HUANG**[1,1], K. KITAMURA[2], M. SCHEBESTA[3], F. SERLUCA[3], P. ZHU[3], T. BOUWMEESTER[4], R. FRIEDRICH[1];
[1]Friedrich Miescher Inst., Basel, Switzerland; [2]Kyoto Univ., Kyoto, Japan; [3]Novartis Inst. for Biomed. Res., Cambridge, MA; [4]Novartis Inst. for Biomed. Res., Basel, Switzerland

Abstract: Many autism spectrum disorders (ASDs) have a genetic basis but it remains unclear how genetic modifications cause disease phenotypes such as social deficits and repetitive behaviors. We examined effects of mutations in ASD-linked genes on social behaviors of adult zebrafish. We focused on shoaling, a social behavior that can be elicited by visual stimuli. Using systematically modified movies, we found that attraction to shoals is modulated by skin pigmentation patterns, body size and aspect ratio. Wild-type fish and various mutants showed similar behavioral response profiles to modified fish movies. However, fish carrying mutations in shank3B and fish expressing a dominant-negative shank3B construct exhibited a sustained behavioral response to repeated stimuli, while responses of wild-type fish attenuated. To

investigate the neuronal correlates of normal and abnormal social behaviors we have established a virtual-reality environment for head-fixed adult zebrafish. This approach will be used to analyze neuronal activity patterns in the forebrain during social behaviors by multiphoton calcium imaging.

Disclosures: **K. Huang:** None. **K. Kitamura:** None. **M. Schebesta:** None. **F. Serluca:** None. **P. Zhu:** None. **T. Bouwmeester:** None. **R. Friedrich:** None.

Poster

652. New Assays for Monitoring Cells, Circuits, and Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 652.08/OOO4

Topic: I.04. Physiological Methods

Title: Pharmacological impacts on cardiovascular output and performance in neurobehavioral tasks

Authors: ***A. L. ZMAROWSKI**[1], M. A. HAWK[1], B. VISNICK[1], S. REED[1], J. KINZER[1], S. ARMENTROUT[1], B. WOOD[1], R. HAMLIN[2], T. M. VINCI[1];
[1]Battelle, West Jefferson, OH; [2]The Ohio State Univ., Columbus, OH

Abstract: The safety pharmacology core battery investigates the effects of pharmaceuticals on vital systems including cardiovascular and central nervous system (CNS) function. CNS evaluation includes sensory/motor reflex responses, coordination and motor activity assessments. This study was conducted to evaluate cardiovascular changes during baseline performance in neurobehavioral tests and after treatment with pharmacological agents. Animals were implanted with telemetry units capable of monitoring blood pressure (BP) and heart rate (HR). After surgical recovery, startle response, rotarod performance and locomotor activity were tested. Rats were treated subcutaneously with amphetamine (AMPH, 2 mg/kg) and MK-801 (0.2 mg/kg) and intravenously with Diazepam (DIAZ, 10 mg/kg) on separate days prior to behavioral testing. Cardiovascular data and behavior were assessed prior to treatment to serve as the control. HR increases were demonstrated with handling and during behavioral tests. In general, HR decreased with habituation to the startle and motor activity tests over time. AMPH treatment impaired startle pre-pulse inhibition and increased locomotor activity. HR was elevated during startle and rotarod performance to the same magnitude as control levels. In the motor activity test, animals showed slight habituation over time though activity remained higher than control levels. HR showed less fluctuation compared to control levels and remained elevated throughout testing. DIAZ reduced HR from baseline, control levels, and reduced performance in both startle and motor activity. HR remained elevated by the loudest stimuli for a longer duration in the startle

task compared to control levels. HR effects were reduced during rotarod testing and had an attenuated HR when initially placed into the motor activity apparatus. HR and BP were markedly increased with performance of all behavioral tests with MK-801 treatment; HR was increased by an average of 78%. However, HR spikes from handling and placement into each testing apparatus were minimized compared to control levels. MK-801 increased the magnitude of the startle response; animals were unable to perform on the rotarod, falling off almost instantly after placement. In the motor activity test, animals were ataxic and uncoordinated, and had a near complete suppression of rearing behavior. No habituation was evident with MK-801 treatment. The combined evaluation of cardiovascular and neurobehavioral testing allow for more comprehensive insight of drug effects in pharmaceutical safety testing.

Disclosures: **A.L. Zmarowski:** A. Employment/Salary (full or part-time): Battelle. **M.A. Hawk:** A. Employment/Salary (full or part-time): Battelle. **B. Visnick:** A. Employment/Salary (full or part-time): Battelle. **S. Reed:** A. Employment/Salary (full or part-time): Battelle. **J. Kinzer:** A. Employment/Salary (full or part-time): Battelle. **S. Armentrout:** A. Employment/Salary (full or part-time): Battelle. **B. Wood:** A. Employment/Salary (full or part-time): Battelle. **R. Hamlin:** None. **T.M. Vinci:** A. Employment/Salary (full or part-time): Battelle.

Poster

652. New Assays for Monitoring Cells, Circuits, and Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 652.09/OOO5

Topic: I.04. Physiological Methods

Title: Applicability of the functional observational battery in canines for safety assessment

Authors: *M. A. HAWK, T. M. VINCI, K. R. HANJORA, J. R. EVANS, S. V. ARMENTROUT, B. J. WOOD, A. ZMAROWSKI;
Safety Pharmacol., Battelle, West Jefferson, OH

Abstract: A functional observational battery (FOB) is typically performed in rodents to assess central nervous system integrity for drug safety. However, dogs and non-human primates are better models for certain drug classes. A comprehensive assay to evaluate central nervous system (CNS) integrity in the dog has not been extensively developed or used. Battelle has validated a series of approximately 32 home cage and open field tests for a rapid CNS screening in dogs. Home cage tests include looking for signs of lethargy, evaluating posture, tremor/convulsive activity, bizarre behaviors and response to handling. Open field testing consisted of evaluating gait, activity (number of squares entered on a floor grid and rearing), vocalization during the test,

as well as their ability find and consume treats in the open field. The wheelbarrow response test and visual placing test were also conducted. Dogs were tested for palpebral reflex, pupillary diameter response, nystagmus reflex response, tactile response, acoustic startle and toe pinch for pain perception. Paw flexor withdraw and limb extensor thrust response were evaluated in each dog and body temperature was collected. It was shown that the FOB could be used successfully to identify and differentiate the behavioral effects of stimulants such as amphetamine (1.5 and 8 mg/kg) and methylphenidate (0.5 mg/kg), and sedative agents such as butorphanol (0.4 mg/kg) and acepromazine (0.55 mg/kg) as compared to dogs treated with sterile water for injection. We have developed this FOB for drug screening in dogs that was based partially on an canine test battery described in the literature (Gad et al., 2003). Consistent with the Battelle rodent and NHP FOB models, data from the present study in Beagle dogs have shown an ability to discriminate the behavioral effects of different drug classes. A dog FOB testing paradigm may be a viable alternative to the NHP model for drug safety evaluation, especially for biologics.

Disclosures: **M.A. Hawk:** A. Employment/Salary (full or part-time): Battelle. **T.M. Vinci:** A. Employment/Salary (full or part-time): Battelle. **K.R. Hanjora:** A. Employment/Salary (full or part-time): Battelle. **J.R. Evans:** A. Employment/Salary (full or part-time): Battelle. **S.V. Armentrout:** A. Employment/Salary (full or part-time): Battelle. **B.J. Wood:** A. Employment/Salary (full or part-time): Battelle. **A. Zmarowski:** A. Employment/Salary (full or part-time): Battelle.

Poster

652. New Assays for Monitoring Cells, Circuits, and Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 652.10/OOO6

Topic: I.04. Physiological Methods

Support: Wellcome Trust 102372/Z/13/Z

Title: A low cost platform for large scale behavioral experiments in mice

Authors: *Y. WEISSENBERGER[1], M. C. KAHN[1], P. KEATING[1,2], A. J. KING[1], J. C. DAHMEN[1];
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Abstract: To understand the neural basis of complex behavior, animals are often trained to perform carefully controlled tasks, which is often difficult, labor intensive and costly. This limits both the complexity of behaviors that animals can be trained to perform, as well as the diversity

of environments learned behaviors are probed in. These factors can impede progress in understanding complex behavior and in turn its neural bases. To avoid these problems, we developed a low cost, high-throughput system for conducting behavioral experiments in mice, which is easy to implement and use, together with associated analysis methods. Our system is based on the Python programming language and the Raspberry-Pi series of micro-controllers. Here, we present the architecture of the system, and proof of concept behavioral data from frequency and level discrimination tasks as well as tone in noise detection. We envision that the platform will prove useful in a variety of domains: training mice to perform complex tasks, fast screening of transgenics on standardized tasks, and systematic exploration of parameter spaces of simple tasks. Together these advantages should allow us to address more effectively the mechanisms underlying learned behavior and thereby their neural bases.

Disclosures: Y. Weissenberger: None. **M.C. Kahn:** None. **P. Keating:** None. **A.J. King:** None. **J.C. Dahmen:** None.

Poster

653. Data Analysis and Statistics: Neuronal Networks

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 653.01/OOO7

Topic: I.07. Data Analysis and Statistics

Title: Functional connectivity analysis during the execution of a willed movement.

Authors: *C. KARMONIK[1], S. H. FUNG[2], M. DULAY[3], R. G. GROSSMAN[4];
[1]Houston Methodist Res. Inst., Houston, TX; [2]Radiology, [3]Neurol., [4]Neurosurg., Houston Methodist Hosp., Houston, TX

Abstract: Background: To identify, using fMRI BOLD activation maps, the brain regions and neural pathways activated during the execution of a willed movement and to quantify the functional connectivity of these regions.**Methods:**fMRI images (TR=1300ms) were obtained from 23 healthy volunteers who viewed alternately a green visual field for 50 seconds followed by a face for 10 seconds. Ten faces were presented serially in random order. Subjects were instructed to squeeze a ball with their right (dominant) hand if they perceived a face as unpleasant. BOLD activation maps were calculated using the Generalized Linear Model and brain regions activated during the execution of the willed movement were identified ($p<0.05$, AFNI software, NIH). fMRI and anatomical datasets were then loaded into the connectivity toolbox (conn, MATLAB) and functional connectivity strength (summed T-values) and number of connections were determined for these regions ($p<0<05$, FDR corrected).**Results**: In all subjects, five faces were perceived as unpleasant and elicited a willed motor response. Brain

regions with highest BOLD activation during the motor response included visual cortex (VCx), precuneus (pCun), thalamus (Thal), lentiform nucleus (Lent), caudate (Cd), cingulate cortex(ACC and PCC), fusiform gyrus (FusG), insula (Ins), supplementary motor area (SMA), sensory cortex (SCx), motor cortex (MCx) and regions in the superior frontal gyrus (SFG) (figure 1A). Functional connectivity for MCx was extensive including VCx, SMA and SCx (figure 1B). Ins and MCx showed highest connection strength (339 and 324, respectively) followed by SCx and VCc (274 and 253, respectively) (figure 1C). Number of connections correlated significantly ($R=0.84, p<0.001$) with connectivity strength for these regions (figure 1D). **Conclusion**: Execution of a willed movement in response to a visual stimulus perceived as unpleasant recruited a widespread network of brain regions. Motor cortex, sensory cortex, SMA, insula and visual cortex exhibited the largest connection strength and the greatest number of connections.

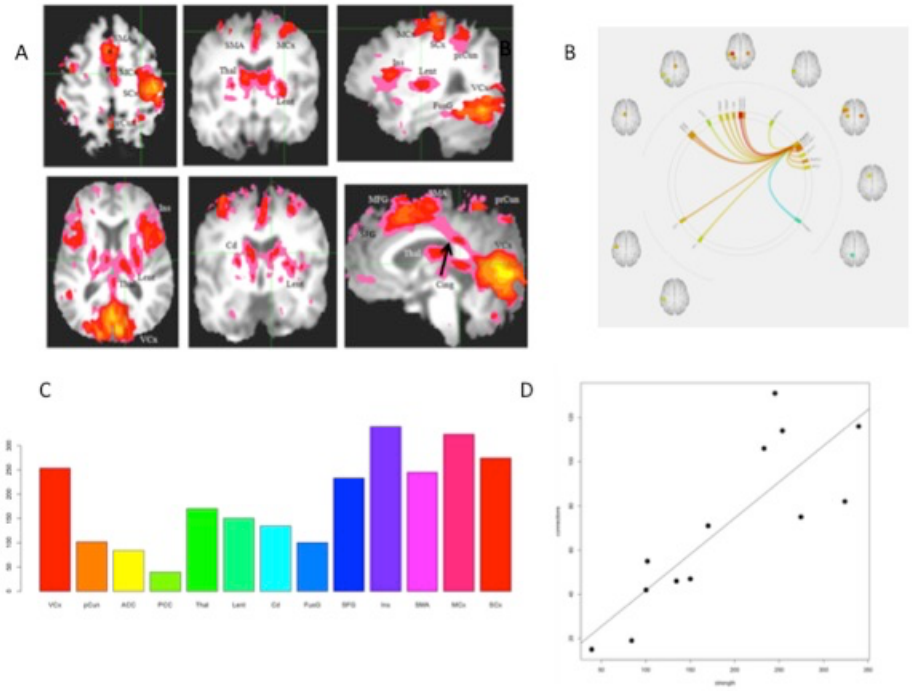

Figure 1: A: BOLD activation pattern for the execution of a willed movement as described in the text. B: Connectivity pattern for the left MCx is widespread including VCx, SMA, SCx and SFG. C: Functional connectivity strength of brain regions displayed in A. Ins, SCx, MCx, SMA and VCx exhibit largest connection strength (expressed by summed T-values). D: Number of connections and connections strength correlate significantly ($R=0.84$, $p<0.001$).

Disclosures: C. Karmonik: None. **S.H. Fung:** None. **M. Dulay:** None. **R.G. Grossman:** None.

Poster

653. Data Analysis and Statistics: Neuronal Networks

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 653.02/OOO8

Topic: I.07. Data Analysis and Statistics

Support: NSFC Grant 61371014

NSFC Grant 61421064

Title: Fiber analysis with scalable tensor covering from micro- to macroscopic level resolution

Authors: ***S. CHEN**, X. LI, A. LI, J. PENG, H. GONG, Q. LUO;
Huazhong Univ. of Sci. and Technol., Hubei, China

Abstract: Neuroanatomical connectivity is essential to understanding brain functions and diseases. Light microscopy and labeling methods have greatly impacted the progress of brain connectome. Quantitative analysis of fiber orientation and structural connectivity at cellular resolution have led to great challenges. Here, principal component analysis based structure tensor (pST) has been introduced for quantifying fiber architecture with tunable scale from micro- to macroscopic level. The pST method has allowed for deciphering fiber density, color-encoded orientation and fiber tractography based on developed software (e.g. DSI Studio). Tested with both Thy1-GFP and virus injection labeling mice datasets acquired with micro-optical sectioning tomography system, the proposed method enabled effective characterization of single axon/dendrite and ensemble of fibers in 3D space or even whole mouse brain. Compared with the conventional Hessian matrix based structure tensor analysis, our method exceled in accurate detection and computation efficacy. Thus, pST would be a helpful tool for fiber quantification and reliable validation of diffusion tensor imaging.

Disclosures: **S. Chen:** None. **X. Li:** None. **A. Li:** None. **J. Peng:** None. **H. Gong:** None. **Q. Luo:** None.

Poster

653. Data Analysis and Statistics: Neuronal Networks

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 653.03/OOO9

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant DC005808

Title: Studying vocal communication of marmoset monkeys (Callithrix jacchus) in a rich, socially-interactive, captive environment

Authors: *L. ZHAO, X. WANG;
Dept. of Biomed. Engin., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Communication behaviors of non-human primates can be considered an evolutionary precursor for human social interactions and may serve as an important behavioral assay to study disorders in human social behaviors such as autism spectrum disorders. The common marmoset (*Callithrix jacchus*) is a highly vocal New World primate species that has emerged in recent years as a promising model system for studying neural basis of vocal communication. Marmosets live in social groups in their natural habitat and maintain a high level of social interactions between individuals in captivity through their vocalizations even. However, studies examining marmoset vocalizations so far have mostly focused on calls produced by individual animals or vocal exchanges between a pair of animals (e.g., antiphonal calling behavior). What has been missing is the characterization of vocal behaviors of marmosets in a richer social environment composed of a group of individuals. In order to fully understand vocal behaviors of marmosets, it is necessary to study call sequences generated by animals living in a social setting and quantify vocal exchanges among multiple animals. To tackle this problem, we have developed a behavior monitoring system in a large breeding colony that is capable of recording and analyzing vocalizations produced by multiple marmosets over a long period of time. By using parabolic microphones and miniature wireless microphones carried by individual marmosets, we are able to detect both loud and quiet calls in a noisy environment and identify individual callers when marmosets are either single-housed or group-housed. Our preliminary data demonstrate that marmosets preferentially communicate with a subset of other individuals through vocal exchanges. Such analyses enable experiments to study social interaction behaviors in this species and the neural substrates for vocal communication in a natural environment.

Disclosures: L. Zhao: None. **X. Wang:** None.

Poster

653. Data Analysis and Statistics: Neuronal Networks

Location: Halls B-H

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Program#/Poster#: 653.04/OOO10

Topic: I.07. Data Analysis and Statistics

Support: Polish Ministry for Science and Higher Education grant 2948/7.PR/2013/2

Hungarian Scientific Research Fund grant OTKA K113147

National Science Centre, Poland, grant 2015/17/B/ST7/04123

Title: Kernel methods for reconstructing current sources of extracellular potentials for single cells, slices, and the whole brains

Authors: *D. K. WOJCIK[1], C. CHINTALURI[1], D. CSERPAN[2], M. B. CZERWINSKI[1], M. KOWALSKA[1], Z. SOMOGYVARI[2];
[1]Nencki Inst. of Exptl. Biol., Warszawa, Poland; [2]Wigner Res. Ctr. for Physics HAS, Budapest, Hungary

Abstract: Extracellular recordings of electric potential are a popular tool for investigations of brain activity on all scales, from single neurons, through populations, to the whole brains, in animals and humans, in vitro and in vivo. The specific information available in the recording depends on the physical settings of the system (brain+electrode). The more selective smaller electrodes are used to capture local information (spikes or local field potential, LFP) while larger electrodes are used for subdural recordings (ECoG), on the scalp (EEG), but also as depth electrodes in humans (SEEG). The advantages of extracellular electric potential are the ease of recording and its stability. Its problem is interpretation: long range of electric field implies high correlations between recording sites. Thus a typical recording reflects activity of many cells, populations and regions, depending on level. To overcome this problem we may reconstruct the distribution of current sources (CSD) underlying the measurement, which is typically done on systems level from multiple LFP on regular grids.
We recently proposed a kernel-based method of CSD estimation from multiple LFP recordings from arbitrarily placed probes (i.e. not necessarily on a grid) which we called kernel Current Source Density (kCSD). In this overview we present the recent advances of this method, latest software implementations, and explain why it works. We also show two recent developments, skCSD (single cell kCSD) and kESI (kernel Electrophysiological Source Imaging). skCSD assumes that we know which part of the recorded signal comes from a given cell and we have access to the morphology of the cell. This could be achieved by patching a cell, driving it externally while recording the potential on a multielectrode array, injecting a dye, and reconstructing the morphology. In this case we know that the sources must be located on the cell and this information can be successfully used in source estimation. In kESI we consider simultaneous recordings with subdural ECoG (strip and grid electrodes) and with depth electrodes (SEEG). Such recordings are taken on some epileptic patients prepared for surgical removal of epileptogenic zone. When MR scan of the patient head is taken and the positions of the electrodes are known as well as the brain's shape, the idea of kCSD can be applied to constrain the possible distribution of sources facilitating localization of the foci.

Disclosures: D.K. Wojcik: None. **C. Chintaluri:** None. **D. Cserpan:** None. **M.B. Czerwinski:** None. **M. Kowalska:** None. **Z. Somogyvari:** None.

Poster

653. Data Analysis and Statistics: Neuronal Networks

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 653.05/OOO11

Topic: I.07. Data Analysis and Statistics

Title: Abnormal metabolic connectivity based on ^{18}F-FDG PET image in Alzheimer's disease mouse model

Authors: *Y. CHOI, H. KANG, K. KIM, D. HWANG, D. LEE;
Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Analyzing the change of functional brain connectivity in cognitive impairment during the course of Alzheimer disease (AD) is essential in terms of development of new network diagnose and therapeutic monitoring for AD. In this study, we developed the diagnostic method to assess metabolic brain connectivity for AD based on integrated brain imaging technologies. To analyze metabolic network, we acquired [^{18}F] fluorodeoxyglucose positron emission tomography (FDG-PET) in the 4, 8, and 12 months old 5XFAD mice which overexpress human APP695 with the Swedish, Florida, and London mutations along with human PS1 harboring two FAD mutations. When metabolic images in AD model were directly compared with those in age-matched control, no regional differences in the brain FDG uptake was observed. Then, persistent brain network homology was applied to investigate metabolic connectivity with a threshold-free approach and the difference between two networks was compared by single linkage distances (SLDs) in all pairwise nodes. Metabolic connectivity significantly decreased between globus pallidus and entorhinal cortex/ perirhinal cortex/ amygdala, amygdala and entorhinal cortex/ perirhinal cortex in the AD mouse. In conclusion, AD mice showed reduced connectivity among memory-related brain regions such as amygdala, entorhinal cortex and perirhinal cortex. These results suggest that this multiscale and threshold-free network analysis could be used to measure abnormal connectivity in the AD animal model as an imaging-based network biomarkers.

Disclosures: Y. Choi: None. **H. Kang:** None. **K. Kim:** None. **D. Hwang:** None. **D. Lee:** None.

Poster

653. Data Analysis and Statistics: Neuronal Networks

Location: Halls B-H

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Program#/Poster#: 653.06/OOO12

Topic: I.07. Data Analysis and Statistics

Support: Simons scgb 325548

NIH RO1 EY024067

Title: A comparison of single and multi-shell diffusion-weighted MRI imaging in the anesthetized macaque

Authors: *K. BROWN[1], P. VELASCO, 10009[2], B. PESARAN, 10009[1];
[1]Ctr. for Neural Sci., [2]Ctr. for Brain Imaging, New York Univ., New York, NY

Abstract: White-matter tractography using diffusion-weighted MRI imaging (DWI) is widely used to study anatomical connections in the primate brain. Recent interest in corticothalamocortical circuits suggests that communication across cortex depends significantly on association thalamus . As a result, correct identification of anatomical pathways depends not only on identifying cortico-cortical tracts, but constituent thalamic relay tracts as well. Advances in multi-shell imaging sequences promise greater accuracy in reconstructing anatomical pathways. However, a direct *in vivo* comparison of single and multi-shell methods for tractography in non-human primate has not been previously reported. Here, we compare the efficacy of single-shell and multi-shell acquisition schemes for *in vivo* DWI tractography. To judge the effectiveness of single-shell and multi-shell acquisition schemes, we reconstructed known thalamocortical projections using a probabilistic tracking method designed to distinguish crossing fibers, segmented the thalamus according to the most likely cortical projection target for each voxel, and compared this classification to known thalamic subdivisions in the Paxinos atlas. Data were acquired with a 3T Siemens Allegra (Erlangen, Germany) using 3 elements out of a 4-channel phased array from Nova Medical Inc. (Wilmington, MA) and 64 gradient directions. To correct for geometric distortions from field inhomogeneities caused by the non-zero off-resonance fields, data was collected with reversed phase-encode blips, forming pairs of images with distortions going in opposite directions. From these pairs the susceptibility-induced off-resonance field was estimated and the two images were combined into a single corrected one. We modeled fiber orientations for each voxel with up to two crossing fibers using a multi-shell model. We then obtained probable paths between the thalamus and each cortical target using FMRIB's probtrackx tool for probabilistic diffusion tractography. For each voxel in the thalamus seed mask, 5000 samples were drawn from the probability distribution given by a resampling procedure on the crossing fibers.
We found that single-shell acquisition underperformed the multi-shell acquisition scheme in

thalamic segmentation. Our results indicate that current *in vivo* approaches to identifying large-scale macrocircuits across macaque cortex for same-subject functional imaging and surgical targeting for electrophysiological recording can be improved using multishell DWI targeting.

Disclosures: **K. Brown:** None. **P. Velasco:** None. **B. Pesaran:** None.

Poster

653. Data Analysis and Statistics: Neuronal Networks

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Topic: I.07. Data Analysis and Statistics

Support: National Natural Science Foundation of China under Grants 81227901, 61231004, 81301346, 81527805, 81501616, 81471739

Chinese Academy of Sciences (Scientific Research and Equipment Development Project YZ201457, Strategic Priority Research Program XDB02060010, International Innovation Team 20140491524, the Key Research Program KGZD-EW-T03)

Title: Brain blood vessel segmentation using local support vector machine

Authors: *H. HUI, C. HU, H. MENG, X. MA, X. YANG, J. TIAN;
Key Lab. of Mol. Imaging, CAS, Inst. of Automation, Chinese Acad. of Scienc, Beijing, China

Abstract: Introduction: Brain vessel segmentation is one of the most important tasks in image analysis for brain vasculature network study. Semi-automatic or automatic blood vessel region extraction approaches can provide useful information for image registration and three-dimensional reconstruction in image post-processing procedure. Generally, classical way is to apply vessel enhancement filtering based on Hessian matrix. However, the design of accurate and efficient vessel segmentation algorithms is still challenging, due to the variety and complexity of images, especially in brain vessel segmentation.
Methods: In this paper, the brain of new-born (5 days) C57BL/6J mouse was immunolabeled or traced tissues were cleared with BABB clearing method. The intact mouse brain was imaged by light-sheet microscope equipped with a sCMOS camera and 2x/NA 0.5, 6mm working distance dipping cap. Vessel Segmentation: In this paper, we present an approach that using the grey level of the pixel, Local binary pattern (LBP) and eigenvalues of Hessian matrix as feature vector. Support vector machine (SVM) was used to classify image pixels as background and foreground, then the outliers were removed to achieve the blood vessel segmentation. We also compared our approach with the segmentation results using Fuzzy Local Information C-Means Clustering

(FLICM) with vessel enhancement filtering. The experimental results illustrated that our approach offer more classification precision than FLICM for acquired blood vessel images by light-sheet microscope.
Conclusion: In this paper, a class-based method by combining the eigenvalues of Hessian matrix, LBP and the grey level of the pixel as feature vector was introduced. The SVM was chosen as a classifier, due to its robustness for small size, high dimensional and nonlinear samples. Only five features of the first four images were used for training sample, we have achieved more than 97% accuracy for vessel segmentation. The accuracy can be increased by adding more features, such as texture and intensity statistics features (Gray level co-occurrence matrix).

Disclosures: **H. Hui:** None. **C. Hu:** None. **H. Meng:** None. **X. Ma:** None. **X. Yang:** None. **J. Tian:** None.

Poster

653. Data Analysis and Statistics: Neuronal Networks

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 653.08/OOO14

Topic: I.07. Data Analysis and Statistics

Title: Dynamic network reconfiguration during a continuous natural stimulus

Authors: ***K. TAN**[1,3], J. LU[1], J. CHEN[2], E. SIMONY[2], H. LIU[1], U. HASSON[2];
[1]Operations Res. and Financial Engin., [2]Psychology and the Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [3]Statistics and Biostatistics, Rutgers Univ., New Brunswick, NJ

Abstract: Recent advances in fMRI analysis techniques have enabled exploration of stimulus-locked network dynamics during complex natural stimuli (Simony et al., 2016). In this new method, correlations of timecourses are calculated between any two locations in the brain, exactly as in standard "functional connectivity"; however, instead of using two locations within the same brain, we use two locations in two different brains of participants viewing or listening to the same naturalistic stimulus (an audiovisual movie or auditory narrative). This modification results in a measure that is free from within-participant intrinsic noise, and locked to the stimulus input. Using this technique, termed "Inter-Subject Functional Correlation" (ISFC), we examine large-scale network dynamics in the brain during a 50-minute audiovisual movie. We assess the impact of adjusting the parameters of window size, dynamic calculation of clustering solutions as the movie storyline unfolds, and implement a mixed-membership clustering technique (Gopalan and Blei, 2013) in order to capture how the sharing of nodes between networks changes over time. All results are cross-validated in two independent groups of participants. We hope that the

development and refinement of these techniques will enhance our ability to understand how networks reconfigure across time in real-world contexts.

Disclosures: **K. Tan:** None. **J. Lu:** None. **J. Chen:** None. **E. Simony:** None. **H. Liu:** None. **U. Hasson:** None.

Poster

653. Data Analysis and Statistics: Neuronal Networks

Location: Halls B-H

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Program#/Poster#: 653.09/OOO15

Topic: I.07. Data Analysis and Statistics

Support: NSFCs 912322015, 81327802, 61008053 and 61205196

Title: Reconstruction of burst activity from calcium imaging of neuronal population via Lq minimization and interval screening

Authors: ***X. LIU**[1], T. QUAN[2], X. LV[2], S. ZENG[2];
[1]Huazhong Univ. of Sci. and Technol., Hubei, China; [2]Wuhan Natl. Lab. for Optoelectronics, Huazhong Univ. of Sci. and Technol., Wuhan, China

Abstract: Calcium imaging is becoming an increasingly popular technology to indirectly measure activity patterns in local neuronal networks. Based on the dependence of calcium fluorescence on neuronal spiking, two-photon calcium imaging affords single-cell resolution of neuronal population activity. However, it is still difficult to reconstruct neuronal activity from complex calcium fluorescence traces, particularly for traces contaminated by noise. Here we propose a reconstruction method that applies Lq minimization approach and interval screening (LqIS). Lq minimization approach is a widely used technique for sparse signal reconstruction. In the designed model for reconstruction, several properties of the AP train including non-negativity, sparsity, and firing rate are considered. The neuronal spike firings could be recovered from the complicated calcium fluorescence trace through Lq minimization. IS, introduced in our previous works, is used to screen out the spike interval so that the reconstruction method can only analyze the calcium trace in these effective intervals. This procedure could largely reduce the dimension of the reconstruction model. Results show that the introduction of IS vastly increases the speed of reconstruction. Compared to some available methods, like L1, Deconv and non-negative deconvolution (NNDeconv), the LqIS is superior in reconstructing neuronal burst activity from Ca2+ traces with low SNR. Moreover, we demonstrate that spikes can be automatically reconstructed from the calcium imaging data of a neuronal population with the LqIS approach.

Disclosures: **X. Liu:** None. **T. Quan:** None. **X. Lv:** None. **S. Zeng:** None.

Poster

653. Data Analysis and Statistics: Neuronal Networks

Location: Halls B-H

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Topic: I.07. Data Analysis and Statistics

Support: Bernstein Award Udo Ernst BMBF Grant 01GQ1106 (Bundesministerium für Bildung und Forschung)

Title: Determining dynamic couplings using Topological Causality

Authors: ***K. PAWELZIK**[1], D. HARNACK[2];
[1]Univ. Bremen, Inst. Theoretical Physics, Bremen, Germany; [2]Univ. Bremen, Inst. Theoretical Physics, Univ. Bremen, Bremen 28359, Germany

Abstract: Effective directed influences among cortical areas vary over time and may even switch their dominant direction. Since local cortical circuits can be considered to be well described by stochastic dynamical systems, previous methods for estimating causal relations that are specialized for either stochastic or deterministic dynamical systems may yield misleading and contradictory results. Here we investigate a mathematically well defined approach for describing causal interactions (by Harnack&Pawelzik, arXiv) that can be used to estimate state dependent causal influences among stochastic dynamical systems from measured time series. We apply the novel method to simulated and to electrophysiological data and demonstrate that it in fact can reliably determine direction and strength of dynamic influences and reveal their time dependence. Comparisons with other methods including Granger Causality (Granger 1969) and Convergent Cross-Mapping (Sugihara et al., Science 2012) for estimating causal influences among deterministic dynamical systems reveal the abilities and limits of this novel method.

Disclosures: **K. Pawelzik:** None. **D. Harnack:** None.

Poster

653. Data Analysis and Statistics: Neuronal Networks

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 653.11/OOO17

Topic: I.07. Data Analysis and Statistics

Support: the program for Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS) from Japan Agency for Medical Research and development, AMED

Title: Neuroinformatics platform for sharing big data beween servers and clients

Authors: *Y. YAMAGUCHI, A. WOODWARD, Y. MORII, M. MAEDA, T. TAKEUCHI, T. HASHIKAWA, H. OKA, Y. OKUMURA;
Neuroinformatics Japan Center, RIKEN BSI, Wako, Japan

Abstract: The program for Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS) aims to integrate heterogeneous big neuronal data of non-human primate, the common marmoset, for comprehensive understanding of neuronal network and its relation with human neuronal diseases. http://brainminds.jp/en/ To conduct this project as collaboration among institutions, an innovative mechanism to share big data on computer networks is needed.
In this purpose, we have developed a neuroinformatics platform. It consists of two parts: one is the research platform with large-scale shared storage, HPC, VDI CLOUD and the other for data portal. In the research platform, every data is registered to and managed as metadata base. Data registration and sharing among users are operated from the research portal of the research platform. For the sake of analysis of the stored data, we designed the platform as an integrative analysis environment where analysis pipeline tools, HPC, VM and storage are directly linked though high-speed data-transfer system. This design enables to avoid troubles in big data transfer between the data server and clients. In this line, we also developed web brain atlas viewer, ZAViewer, for viewing heterogeneous images and python-base pipeline tool, SPyKGD, connecting the storage, HPC and VM. This design of the platform and tool development shall be veneficial in big data analysis not only in Brain/MINDS but also in large-scale brain projects.

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Poster

653. Data Analysis and Statistics: Neuronal Networks

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Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 653.12/OOO18

Topic: I.07. Data Analysis and Statistics

Support: BIG Seed Award (UChicago)

Title: Analyzing functional connectivity across development in zebra finches

Authors: *K. E. SCHERTZ, E. A. LAYDEN, S. E. LONDON, M. G. BERMAN;
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Abstract: Investigations into the dynamics and consequences of whole-brain resting-state functional connectivity (FC) have provided insights into complex neural processes. In humans, such approaches have highlighted how FC changes across the brain during different cognitive and emotional states (Berman et al., 2014). Here we aimed to understand the mechanisms by which neural maturation and experience converge to construct cognitive circuits during development, thus guiding behavior. Specifically, we implemented resting-state fMRI FC analyses on the zebra finch songbird, a model organism of vocal learning. Male zebra finches learn to sing via social interactions during a sensitive period of development, much as children acquire speech. We hypothesized that unique FC signatures would be evident during key developmental time points in which tutor song memorization becomes possible. Therefore, we acquired structural and functional MRI scans for six male zebra finches, including three birds recorded longitudinally at key time points (day 25, day 45, and day 65 post-hatch) in addition to three fully developed adult males. We used Advanced Normalization Tools (ANTs; Avants et al., 2011) to construct a custom, group-average structural template and to perform tissue segmentation and normalization. Denoising steps were completed using CONN Toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012). We conducted modularity analyses using the Brain Connectivity Toolbox (Rubinov & Sporns, 2010). Analyses for the group-averaged network identified an optimal modularity partition of six modules, which demonstrated a primarily anterior-posterior organization, as well as a pallial-subpallial organization. We compared within- and between-module FC across age groups. Regression analyses revealed increased within-module FC for module 6 ($p = .02$), a largely subpallial brain area, as a function of increasing age. Additionally, between-module FC was increased with age for modules 4 and 6 ($p = .003$), 3 and 4 ($p = .03$), and 4 and 5 ($p = .03$). These results parallel prior findings of age-related increases in modularity in humans. A larger sample size will enable us to investigate the finer scale dynamics occurring at individual developmental time points and how these relate to successful song learning. We also conducted a follow-up investigation using partial least-squares regression to predict voxel-wise functional connectivity strength via age group. This analysis

revealed a significant first latent variable ($p < .001$), wherein adult males showed increased FC in the dorsal pallium relative to the three juvenile developmental time points that parallels our modularity results.

Disclosures: **K.E. Schertz:** None. **E.A. Layden:** None. **S.E. London:** None. **M.G. Berman:** None.

Poster

653. Data Analysis and Statistics: Neuronal Networks

Location: Halls B-H

Time: Tuesday, November 15, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 653.13/OOO19

Topic: I.07. Data Analysis and Statistics

Support: RO1 NS42617

R01 NS085272

Title: detection of ischemic penumbra during acute ischemic stroke injury using perfusion diffusion mismatch as assessed by 9.4T magnetic resonance imaging

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Abstract: Stroke is the fourth leading cause of death in the United States. Advances in magnetic resonance imaging (MRI) have improved diagnostic assessment of stroke-affected brain involvement where accurate quantification is believed important in diagnosis and monitoring therapeutic options. The ischemic penumbra represents a region of hypo-perfused and functionally impaired but viable brain tissue with potential for recovery. The identification of penumbra necessitates measuring areas of reduced blood flow and lesion volume where MR perfusion-diffusion mismatch provides a measure of tissue at risk. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Ohio State University. C57/BL6 mice (n=6) underwent middle cerebral arterial occlusion (MCAO) surgery. MRI was performed 30 minutes after permanent MCAO on a 9.4T magnet. Localizer scans, local shimming using Fastmap and DWI images were acquired using a spin-echo sequence. A method based on the flow-sensitive alternating inversion recovery (FAIR) and a fast rapid acquisition with relaxation enhancement (RARE) were used to acquire images. Perfusion-diffusion mismatch method was used to identify the ischemic penumbra in animals maintained on medial air (room air, RA) during stroke and those maintained on 100% oxygen (supplemental oxygen,

SO) during stroke. Importantly, SO is known to protect brain tissue from stroke-induced injury. At the acute time point of acquisition (30min post-occlusion), T2-weighted MR imaging was unable to resolve stroke-induced injury. Conversely, DWI was effective in quantitatively defining edema at this early time point in the stroke-affected brain. In post processing DWI images, thresholds were applied to delineate hyperintense regions indicative of stroke-induced injury. Compared to RA conditions, SO significantly increased the volume of the ischemic penumbra as evidenced by decreased DWI deficit without change in perfusion deficit. In the setting of ischemic stroke where "time is brain", rapid identification of the ischemic penumbra may prove critical to optimize therapeutic intervention and delivery strategies. While the clinical utility of perfusion-diffusion mismatch remains to be realized, we demonstrate in a pre-clinical setting that PWI coupled with early stage infarct quantification from DWI enables acute determination of the ischemic penumbra.

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